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PHYSIOLOGICAL STUDIES ON CROSSING OVER

by

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The undersigned certify that they have read, and recommend to the Faculty of Graduate Studies for acceptance, a thesis entitled "Physiological Studies on Crossing Over", submitted by Keh-Ping Ting, B.Sc., in partial fulfilment of the requirements for the degree of Doctor of Philosophy.

ABSTRACT

The present study examines the importance of protein and its possible role in the process of crossing over. From a preliminary study of the effects of sodium selenate on barley it was concluded that selenium treatment resulted in a significant reduction in crossing over. Cytological examination of the meiotic cells of treated plants suggested that the reduction of crossing over is not associated with a failure of homologous pairing, but rather with a deformation of the chromosomes.

A study of the effects of selenocystine and selenomethionine on interference in crossing over *Drosophila melanogaster* revealed that:

1. The intensity of reduction of recombination with selenocystine treatment differed among segments in the chromosome; the reduction was greater in the middle segment than in the distal one.
2. Increasing concentrations of selenocystine resulted in consistent decreases in the coefficient of coincidence.
3. An unexpected increase in both recombination fraction and coincidence coefficient occurred at low concentrations of both treatment chemicals.

The observations support the proposition that strand-breakage represents an initial stage of the crossing-over process and that it is mediated by stress on the DNA associated protein of the chromosomes through disulfide-bonding. It is suggested that selenium substitution may affect crossing-over through a change in the stress occasioned by differences in the nature of the diselenide and disulfide bond.

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INTRODUCTION

In 1912 Morgan introduced the term "crossing over" to describe the phenomenon of the recombination of linked characters. Several workers put forward theories to explain the process in the subsequent five decades. More recently these theories have been profoundly affected by the identification of DNA with the genetic material and by the advent of interpretations of biological structure and function at the molecular level. At present, the question: "What is the mechanism underlying crossing-over?" is a highly controversial one, since our knowledge of this process is far from complete. The primary hindrance to current advances in our knowledge of the process lies in the fact that the ultrastructure of the chromosome, as a nucleoprotein aggregate, is not understood. In addition, the conclusions from carefully designed studies are in many instances rendered ambiguous by the discovery of intragenic crossing over, exchange of different segments of a gene-locus, which shows certain features not associated with intergenic crossing over.

The significance of an understanding of this molecular structure in the solution of genetic phenomena is attested to by the extent to which the molecular basis of mutation, replication, and information coding are now understood. It is therefore natural to expect that, since the exchange of genetic material involved in crossing over is closely related to these processes, the knowledge of the molecular structure of the chromosome may be a necessary prerequisite to the understanding of the mechanism of crossing over.

Intragenic crossing over, as it is found in some fungi, shows three specific features. These are negative interference, the tendency for

several exchanges in a locus to occur in a single meiocyte; non-reciprocality, the production of only one of the two reciprocal exchange strands by a single meiocyte; and polarity, the aberrant segregation of hybrid asci for pairs of allelic mutants in a gene locus leading to spore pair segregations wherein the mutant to the one side, that to the other, and the wild type are always present in the ratio 2:1:1 respectively. In intergenic crossing over, on the contrary, the process shows positive interference, the tendency for only one exchange to occur in a segment of given length; reciprocality, the production of both reciprocal-exchange strands; and non-polarity.

Recently advanced hypotheses attempt to reconcile these differences and to correlate them with the molecular data. It is perhaps significant that some of these recognize the need for considering the process as consisting of two distinct phases: a breakage phase and a rejoining phase. It may also be significant that, although these explain the rejoining phase, they rely on earlier ('pre-DNA') theories to explain the breakage phase. Such theories and their factual bases may therefore prove as pertinent as the modern ones to a clear understanding of crossing over.

I. Intergenic Crossing Over at Meiosis

The basic facts about intergenic meiotic crossing over have been summarized by Westergaard (1964) as follows:

1. "It is a result of actual exchanges between homologous chromosomes (Creighton and McClintock 1931; Stern 1933)".
2. "Exchange takes place between chromatids and only two of the four chromatids of a bivalent pair are interchanged in each crossing-over event. Consequently crossing over must take place during or after chromosome replication. (Drosophila, Anderson 1925;

Emerson 1953; Neurospora, Lindegren 1933; Strickland 1961; Perkins 1962 a, b; Aspergillus, Strickland 1958; Chlamydomonas, Ebersold and Levine 1959)".

3. "Crossing over is reciprocal and symmetrical i.e. each crossover gives a tetrad containing two parental non-crossover chromatids and two complementary crossover chromatids".
4. "Classical crossing over is associated with positive interference. The distribution of crossover events is not a random one, because one crossover reduces the probability of a second crossover in the same chromosome arm. Positive interference does not extend across the centromere" (Muller 1916; Mather 1933; Darlington 1937).
5. "It occurs in all organisms with nucleoprotein chromosomes in which genetic studies have been made".

The suggestion that crossing over resulted from strand-exchange (viz. an actual breakage of homologous strands followed by an exchange reunion) was originally proposed by Janssens (1909). This suggestion was embodied in his chiasmatype theory, in which it is postulated that a strand exchange occurs at the four chromatid stage, which, in addition to a recombination of genes, results in the appearance of a cross-shaped configuration, visible at diplotene, called a chiasma. This remarkable theory, except for its suggestion regarding the cause of breakage (a mutual erosion or penetration of contacting strands) has satisfactorily explained all the cytological and genetic phenomena of meiosis that are available for testing the validity of such theories.

Westergaard in his review (1964) states: "one of the most impressive observations in its support is the correlation between crossover suppression and achiasmatic meiosis in those insects where crossing over is sex-restricted", and quotes evidence from the silkworm (Maeda, 1939), Drosophila

(Cooper 1949 and Ohno, et al. 1957) and Phryne (Bauer, 1946 and Wolf, 1950). He draws attention to the reported absence (Meyer 1960) in Drosophila males of the 'synaptinernal complex' at pachytene by Moses (1960) as supporting 'the notion that there is a profound chromosome structural difference between normal meiosis and achiasmatic meiosis'.

Several variations of the chiasmatype theory, which refer only to the mechanism and time of strand exchange and hence to its relationship to chiasma formation, have been proposed. The most important of these is the two-plane hypothesis of Sax (1932). According to this hypothesis an initial separation of the four strands into two strand-pairs immediately follows synapsis. This separation is of such a nature that the associated threads of each pair may be sister-strands in one region and non-sister strands in an adjacent one.

Hence the point between two such regions would be a chiasma and might necessitate breakage to allow separation of the homologues. Certain phenomena such as the terminalization of chiasmata do not support this hypothesis and the original version, under the name of 'partial chiasmatypy' (Darlington 1934, 1935, 1937), prevails.

Regarding the question of the manner by which strand exchange may take place two general hypotheses have been advanced.

The following is a description of the torsional hypothesis of Darlington (1935, 1937) provided by Swanson (1957).

"The torsion hypothesis considers that the two homologues are relationally coiled about each other. Being undivided, they are attracted to each other as a pair to satisfy a two-by-two association. .... However, when the new chromatids are formed, for the two-by-two association will now be between sister chromatids rather than between homologues. The attraction

between homologues is replaced by a repulsion which forces them apart, but since they are relationally coiled about each other, a torsion is developed which eventually breaks two non-sister chromatids. The relational coiling is undone and the torsion relieved by the movement of broken chromatids, and when the broken ends rejoin, if the rejoining is between non-sister strands, a crossover occurs".

Swanson stressed the fact that the basis of Darlington's explanation of the cause of the breakage of strands, viz. 'electrostatic forces of attraction and repulsion', have not been experimentally demonstrated.

For this and other reasons, the Belling hypothesis (1931, 1933) has been favoured in latter years. According to this hypothesis, during the period of close pairing, chromosome strands are built up alongside existing strands by a two part process, in which chromeres are formed opposite existing ones, and are then joined by inter-chromomere fibres. Strand exchange is accomplished by the establishment of inter-connecting fibres between non-sister chromomeres, favoured by relational coiling.

The replication hypothesis implies in the first place that no breakage-reunion takes place in the process, and secondly that chromosome replication and crossing over must coincide in time. Therefore chromosomes must replicate conservatively and should do so during pachytene, since the leptotene chromosome is undivided. It is now certain, as a result of the work of Taylor (1957, 1958), that strand-replication occurs at the last premeiotic interphase rather than at pachytene of meiosis in higher plants. Moreover Taylor (1958) and Sueoka (1960) have demonstrated that chromosome replication follows a semiconservative and not a conservative pattern. The Belling hypothesis appears therefore to be no longer tenable.

II. Intergenic Crossing Over in Relation to Chromosome Structure.

Since the process of crossing over must involve either the separation and reassociation of two homologous DNA molecules at a precise point on each, or an equally precise dissociation and reassociation of linkers from their associated DNA molecules in the homologues; it should be expected that, at some part of the process at least, a rather precise molecular process would be involved. Although several molecular models have been devised to explain crossing over, most of them lack conviction because they assume certain as yet unproven conditions of chromosome structure to be true.

Since crossing over is the result of actual exchanges between two of four homologous chromatids, a molecular process for crossing over, in the first place, requires a very precise understanding of the molecular structure of the chromosome. In prokaryotes the accepted molecular model of the chromosome is rather simple, viz. a ring consisting of uninterrupted DNA or RNA double or single helices. But in eukaryotes, which have nucleoprotein chromosomes as carriers of genetic information, the molecular structure of these chromosomes remains an enigma. It is known that they are built up of DNA and protein macromolecules. The constituent DNA is generally believed to be present in the double helical structure. The chemical nature of the protein constituents is an uncertain matter. Histones (Swift 1962) or histones in combination with other proteins (Peacocke and Drysdale 1965) are thought to be involved. But regarding the specific structural roles played by the histone and non-histone chromosomal proteins in relation to DNA only tentative information is available (Swift 1962).

Molecular models of the chromosome proposed by different workers differ largely in regard to the number of DNA double helices present, and whether the DNA is a single long molecule or several short ones joined by

protein or other links. Taylor (1963) described several models with protein links. Other models have been devised by Freese (1958), Lissouba et al. (1962) to explain their observations suggesting copy choice and polarized segregation respectively. Uhl's model (1964) is based on theoretical considerations. The validity of these models has suffered considerably from the evidence obtained in 1961 by Davison and Levinthal that the chromosome is probably a single continuous strand of DNA. Callan's work (1963) suggested that in newts the meiotic chromosome is a single DNA molecule. Moreover, considerable controversy exists over whether the chromosome is in fact single stranded (Callan 1963), or multistranded (Taylor 1963; Peacocke and Drysdale 1965).

The semi-conservative type of replication of DNA (Meselson and Stahl 1958) and of chromosomes (Sueoka 1960; Taylor 1958) strongly favours the breakage-reunion or chiasmatype crossover and rejects the replication theory, which is possible only if the chromosome shows conservative replication. It should be pointed out, however, that although most fungal geneticists believe chromosome replication in the fungi is semi-conservative, some still favour a conservative mechanism of meiotic DNA replication where there is no evidence of third division segregation (Stadler and Towe 1963). This mechanism of DNA replication would allow the possibility of a copy-choice or replication type crossover. The copy-choice theory relates strand exchange to DNA synthesis: the two events must coincide in time. Cytochemical studies (Taylor 1957, 1958) and data on chromosome breakage by irradiation (Mitra 1958) have shown undoubtfully that in higher plants, e.g. Lilium, chromosome replication takes place at the last premeiotic interphase, whereas it is generally accepted that crossing over occurs at pachytene. Therefore the replication theory of crossing over appears untenable.

III. Intragenic Crossing Over.

In the past decade, new techniques have been developed for studying the fine structure of the gene at the molecular level. The work, initiated by Banzer (1955) with bacteriophage, has been adopted to other micro-organisms (for review see Pontecorvo 1958). The classical concept of the gene as the unit of crossing over has suffered as a result of these studies, which involve intragenic recombination. The three phenomena not found in intergenic crossing over and referred to earlier (viz. negative interference, non-reciprocal crossing over, and polarized crossing over) are obviously peculiar to intragenic crossing over.

In order that the special difficulties in fitting hypotheses of crossing over to these phenomena may be fully appreciated, a more detailed description of these effects after Westergaard (1964) is given below:

"1. Negative interference. If the chromosomes are properly marked it is possible to study the correlation between allelic crossing over and recombination of outside markers. According to the theories of crossing over so far discussed one would expect allelic recombination always to be associated with crossing over between closely linked outside markers. It has been found, however, by Prilchard (1955) that this expectation is not fulfilled. One explanation for this lack of correlation is the occurrence of multiple crossovers within a very short chromosome segment; the phenomenon has therefore been called 'negative interference' by Prilchard. Since it may also have other explanations, the term negative interference is in a way unfortunate because it implies an explanation that may not be correct".

"2. Non-reciprocal crossing over. Since allelic crossing over

is a very rare event (with a probability of 1×10^{-4} or less), the number of crossover tetrads that can be analysed in crosses between biochemical mutants is very small. Even with this low number of tetrads it has been established that allelic crossing over is very often asymetrical and non-reciprocal. A non-allelic-crossover tetrad would be expected to contain both parental types together with the wild type and the double-mutant recombinants. However, in some crossover tetrads a parental type replaces either the wild type recombinant or the double-mutant, and hence is present twice. Such non-reciprocal tetrads were first discovered in yeast by Lindegren (1953) and in Neurospora by Mitchell (1955, 1956), (ref. Roman 1963). The phenomenon has also been called gene conversion, or trans-replication".

"3. Polarized crossing over. The Ascobolus system which involves crosses of a very large number of mutants with white spores and a subsequent scoring of asci in which black (wild-type) spores segregate, led to the discovery of polarized crossing over (Lissouba et al. 1962; Makarewicz 1961). In certain allelic crosses all crossover tetrads are non-reciprocal and it is always the same parental type which is represented twice in the tetrad. A number of mutants which by a linkage analysis can be arranged in a linear order give only non-reciprocal tetrads. It can be shown that there is a left to right (polarized) asymmetry in such crosses; it is always the parental mutant to the one side which is represented once. The term 'polaron' has been coined by Rizet to describe a group of mutants which, when intercrosses, always give non-reciprocal, polarized crossover tetrads. Mutants belonging to different polarons may sometimes give both reciprocal and non-reciprocal crossover tetrads, and sometimes reciprocal crossover tetrads only. Polarized allelic crossing over has also been found in Neurospora (Murray 1963)

and in Aspergillus (Siddiqi 1962; Siddiqi and Putrament 1963)".

Intragenic crossing over associated with one or more of the three characteristic phenomena have been found to occur in many organisms (*Bacteriophage* T4, Chase and Doerman 1958; *Drosophila*, Hexter 1963; Maize, Nelson 1962, see Westergaard, 1964 for review). It would appear that processes common to such widely divergent organisms should not be attributed to accidental qualities of species, but rather to universal feature of these organisms.

A number of new hypotheses have been proposed to reconcile the observations associated with intragenic and intergenic crossing over. As stated by Westergaard (1964) these hypotheses are all, in a sense, modifications of Belling's replication crossing over hypothesis, specified in terms of the molecular structure of DNA and most of the new hypotheses are variants of the so-called copy-choice theory, originally proposed by Lederberg in 1955. The central postulate of this hypothesis and all its variants (Freese 1957; Prilchard 1960 a, b; Stadler 1959 a, b; Stadler and Towe 1963) is that homologous chromosomes are paired, at least at certain points along their length, at the time of replication and that the new strands are replicas of one parental strand in one region and of the other one in another region. To account for polarity, Lissouba et al. (1962) postulate that the direction in which replication occurs is from 'left' to 'right'; (viz. towards sites of a group in which gene conversion occurs more frequently), and that a copying 'error', once initiated, continues to the end of the group of sites, termed a polaron.

Besides the criticisms of the copy-choice hypothesis mentioned earlier (viz. incompatibility with semi-conservative replication and with the observation that DNA replication takes place some time before crossing over), an additional objection, specific to the polaron concept, may be made.

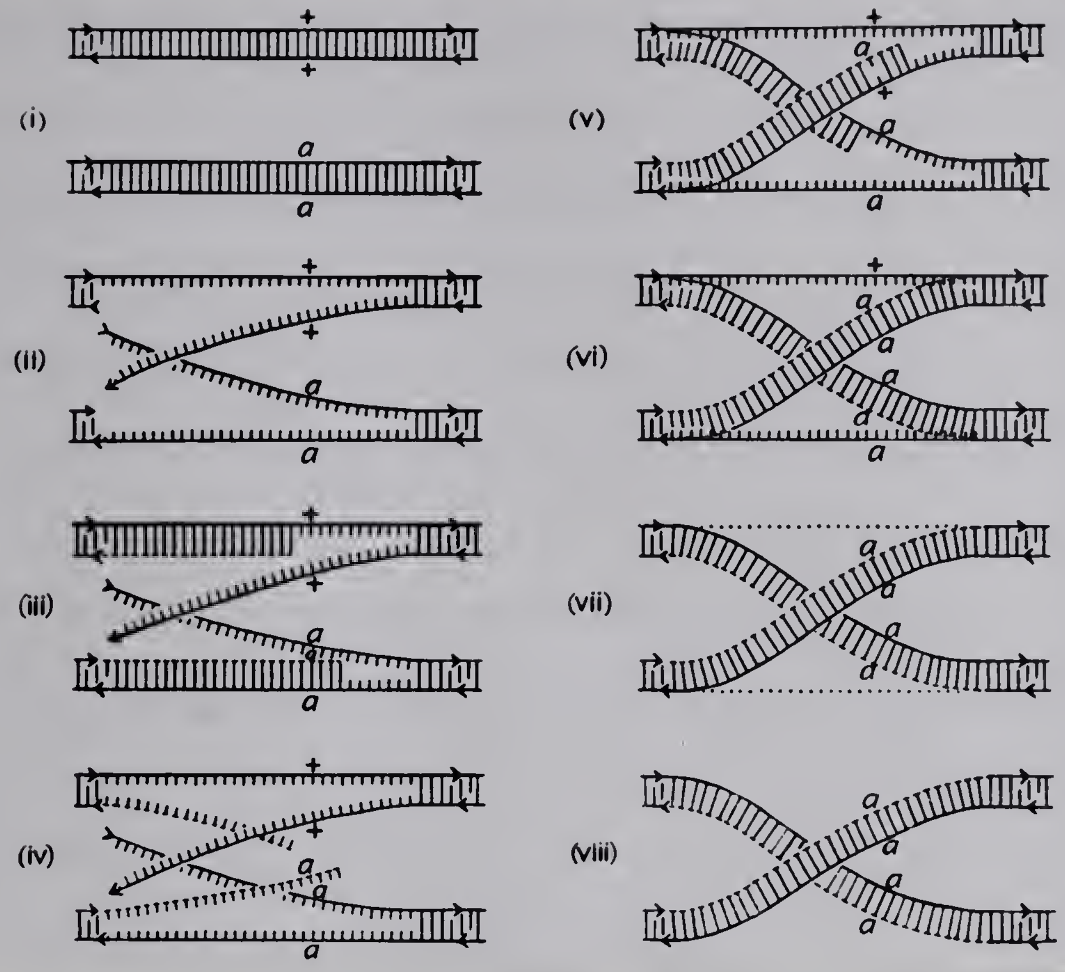
Thus the theory of the polaron demands that few, if any, of the copying errors continue through the intervening 'linkage structure' to the next polaron; whereas Stadler (1963) has shown that, according to Lissouba's own data, a majority do so.

The proposal that intragenic crossing over is associated in point of time with the replication synthesis of DNA at meiosis is not substantiated by unquestionable evidence. In *Saccharomyces*, Sherman and Roman (1963) found evidence for two types of intragenic crossing over, one occurring before and the other after meiosis. The preliminary data suggested that the premeiotic intragenic crossovers coincided with chromosome replication, as indicated by the uptake of tritiated thymidine. However, this substance is preferentially incorporated into RNA in *Neurospora* (Fink 1963; Fink and Fink 1963 a, b), and thus the data of Sherman and Roman may be based on RNA, rather than DNA synthesis.

Some workers have proposed alternatives to the replication hypotheses, which they find unacceptable. Notable among these alternatives are those postulated by Whitehouse (1963), Holliday (1964) and Uhl (1965). The Whitehouse hypothesis appears compatible with all the known characteristics of crossing over.

Whitehouse postulates that crossing over takes place at pachytene by a process of breakage and repair of two non-sister chromatids, each containing one double helix of DNA (see text - figure 1). Breakage consists of a discontinuity in only one strand of the double helix of each chromatid; the points of breakage in the two homologous chromatids need not be strictly at the same genetic site, although some degree of closeness is required for repair.

The processes of repair consist of (a) the synthesis of a comple-



Text figure 1.

The mechanism of crossing-over (after Whitehouse 1965) according to the polaron hybrid DNA model. The lines with arrow-heads represent nucleotide-chains, with the arrows showing the direction of the phosphate-sugar backbones. Broken lines represent newly-synthesised chains, and dotted lines chains which are breaking down. The arrow-heads are placed at the junction of a polaron with neighbouring polarons. Transverse lines extending from one nucleotide-chain to another indicate hydrogen bonding between complementary bases. The letter a indicates the site of a mutation, and the plus sign its normal allele.

ment to each of the unbroken half-helices, and a subsequent unwinding of each synthesised 'end' back to the break point, and (b) the annealing of the broken half-helices of non-sister chromatids to produce continuous crossover double helices, which, in the region adjacent to the break consist of hybrid DNA (see text - figure 1).

The Whitehouse' hypothesis may be used satisfactorily to account

for all the phenomena associated with intergenic crossing over. It may also be used satisfactorily to explain the phenomena associated with intragenic crossing over, if two further assumptions are made; viz. (a) that when the hybrid DNA regions differ in base pattern, a DNA-repair mechanism similar to that found for the repair of UV radiation damage to either the wild-type or to the mutant pattern may occur, and (b) that at certain sites, corresponding to the ends of polarons, the unwinding of the broken ends is stopped.

Some cytochemical evidence has been reported recently in supporting Whitehouse' proposal of localized DNA synthesis at pachytene. In addition to the main DNA synthesis which takes place at premeiotic interphase, a slow DNA synthesis is found to occur at pachytene in the male newt _Triturus viridens_ (Wimber and Prensky 1963). This lagging DNA synthesis at pachytene might be the 'repair synthesis' suggested by Whitehouse.

IV. _The Effects of Various Treatments and Conditions on Crossing Over_.

A number of kinds of treatments and conditions have been found to have an influence on the apparent rate of crossing over in organisms with nucleoprotein chromosomes. A significant effect of some of these on meiotic crossing over is well established (temperature, chromosomal aberrations and several chemicals); with others (ionizing and non-ionizing radiations) no clear cut effect has been found. In general, this treatment approach has proven disappointing from the point of view of those interested in an understanding of the mechanism underlying crossing over. Nevertheless, some contributions have been made in this direction, and a number of specific effects have been discovered that may prove to be significant to an understanding of the process.

1. _Temperature effects_. An enhancing effect of increased temperature

on the rate of intergenic crossing over has been reported for a number of organisms, including Drosophila (Plough 1921), Ustilago (Huttig 1931, 1933) and Ascobolus (Lissouba et al. 1962); a single exception is found in Habrobracon, for which negative results are reported by Clarck (1943). The observation by Plough (1921) that the regions sensitive to temperature are confined to the proximal segments of the second and third chromosomes, which are heterochromatic, suggests that the induction of crossing over in heterochromatic regions might be entirely or almost entirely responsible for the enhancing effects. If so, intragenic crossover frequencies might not be affected; such a situation appears to be the case in Ascobolus where intergenic and intragenic crossing over are differentially affected by temperature (Lissouba et al. 1962).

2. Chromosomal anomalies. In Drosophila it has been found that in individuals heterozygous for inversions and other structural anomalies, the rate of intergenic crossing over in chromosomes not involved in the anomalies is higher than in normal diploid individuals (Dobzhansky 1931; Sturtevant and Beadle 1936; Steinberg and Fraser 1944; Carson 1953; Cooper et al. 1955; Hart and Sandler 1961; Suzuki 1961, 1962; Ramel 1962; Roberts 1962). A representative study of this interchromosomal effect is that of Suzuki (1961, 1962). In this study, various rearrangements of the X-chromosome were found to increase recombination in chromosome III of Drosophila. The regions sensitive to this effect, like those sensitive to high temperature, coincide with the heterochromatic segments proximal to the centromere. Although several hypotheses have been formulated to explain these results (Mather 1936; Schultz and Redfield 1951; Oksala 1958; Suzuki 1962), no one mechanism has been found to have general applicability.

3. Chemical treatments. The earliest, and yet the most extensive

study of chemical effects on the rate of intergenic crossing over was made by Huttig (1933) with synchronized meiotic cultures of Ustilago in which crossing over between the centromere and the mating locus was detectable in single promycelia. Huttig's observations are summarized below:

(a) Ammonium nitrate, thiocyanate and sulfate, despite the differences between the basic anions, give identical crossover frequency versus concentration curves, and all three show the same optimal concentration, above or below which crossing over decreases. The crossover frequency curves obtained with treatments of ammonium chloride, bromide and iodide on the other hand lack regularity, although the three salts exhibit a rather close optimal concentration with respect to crossover frequency.

(b) Potassium and sodium cations at high concentrations enhance crossing over, the former showing an optimal concentration for this effect.

(c) Urethane derivatives all markedly enhance crossing over. The effect increases directly with the number of carbon atoms in the addition group according to the series: methyl-, ethyl-, propyl-urethane.

The presence of concentration optima of various anions and cations for the recombination increase revealed by Huttig's data strongly suggest that non-allelic crossing over in Ustilago is a physiologically controlled process. The enhancing effects of urethane and sodium cations have not been explained.

With the exception of conflicting reports of duplicate experiments (Eversole and Tatum 1956; Levine and Ebersold 1958), proclaiming and denying, respectively, an enhancement of intergenic crossing over in Chlamydomonas by

the metal-chelating agent ethylene diamine tetraacetic acid, no further reports on ion effects are available.

Two clear cut cases of an effect by biologically active organic compounds have been demonstrated. The first is from recent reports that the antibiotic Actinomycin D causes an increase in intergenic crossing over in *Drosophila melanogaster* (Suzuki 1963, 1964). The observation that the region which is sensitive to Actinomycin D lies within the heterochromatic segments near the centromere (Suzuki and Parry 1964) is of great interest, since it parallels the observations described earlier regarding the effects of temperature and of chromosome rearrangements. Furthermore, since this substance has been shown to inhibit DNA-dependent RNA synthesis, the authors conclude that its effect on crossing over is an indirect one, probably the result of a change in the structure of the heterochromatic segments that allows their pairing and crossing over. The second case is from recent demonstrations that some of the mutagenic base analogues enhance intergenic crossing over in *Neurospora* (Wolff and De Serres 1963; Threlkeld 1963). The functions of these agents in altering crossing over frequency are not known. It may be inferred, however, that their effect is related to their ability to substitute for bases in the DNA, and thus to increase the probability of DNA strand breakage or to effect crossing over at the expense of restitution.

4. *Irradiation*. Radiation effects on meiotic recombination in higher organisms have been claimed by many workers (ref. Westergaard 1964). All, however, admit to reservations about their conclusions, and propose further study. It may be noted in passing that both ionizing and the non-ionizing radiations are reported to have a strong effect on somatic crossing over in *Drosophila* (ref. Whittinghill and Davis 1961), Aspergillus (Morpurgo 1962; Kafer 1963) and *Ustilago* (Holliday 1961).

V. Rationale Leading to the Present Experiments.

The present studies are concerned with meiotic intergenic crossing-over of higher organisms. The chiasmatype (or breakage-reunion) theory, despite its failure to account for all the phenomena associated with intragenic crossing over, nevertheless explains satisfactorily all the cytological and genetic phenomena of intergenic crossing over in organisms with nucleoprotein chromosomes. The cytogenetic evidence in support of this theory has been well summarized (refer to page 2) and will not be repeated here. Moreover, it seems premature at present to speculate whether or not intra- and intergenic crossing over are different processes or are aspects of the same process, since our knowledge of their relationship is far from complete.

Breakage-reunion may therefore be considered to be the most tenable hypothesis to explain intergenic crossing over, and hence it may be considered profitable to enquire into the cause of strand breakage and reunion, which undoubtedly would ultimately involve a breakage and reunion of DNA molecules, or, less probably, of protein linkers. The possibilities currently recognized as a cause of breakage are

(1) that it is mediated by enzymes,

(2) that it is mediated by stress.

The present experiments were designed to determine whether protein is involved in crossing over and if so to explore its possible role.

EXPERIMENTAL STUDIES

I. A Study of the Effects of Selenium Treatment on Intergenic Recombination in Barley

Sulphur is a constituent of most proteins, but is absent from DNA molecules. Selenium may be considered an analogue of sulfur to the extent that it can substitute for sulphur in a polypeptide chain by incorporating into such amino acids as cysteine, cystine and methionine to form the corresponding seleno-amino acids. The selenohydryl group is, according to the rule of decreasing reactivity with increasing atomic mass, probably chemically less reactive than the thiohydryl group and therefore more stable. The physico-chemical properties of the structural and metabolic proteins would likely be affected by this substitution but the author has to date, been unable to find any information on this.

Selenium substitution might, however, be expected to disturb the disulfide bonding relationships of chromosomal protein and hence its tertiary structure. Consequently, the shortening, condensation and coiling of chromonemata may be hindered in the early stages of meiosis. Torsional forces, which build up during chromosomal condensation and coil-formation, might then decrease and, if such forces play a part in crossing-over, as suggested by Darlington (1935, 1937), selenium substitution would probably reduce the rate of crossing-over.

On the other hand, the possibility remains that certain specific enzymatic proteins may be responsible for chemical reactions involved in the process of crossing-over. It is well known that the intrachain disulfide bonds of an enzymatic protein play an important role in the determination of its structure and activity. Seleno-sulfur substitutions might therefore inactivate such an enzymatic protein by virtue of changes

in its internal structure. Again a change in crossing over rate would be expected with this substitution, although this might not necessarily be a reduction. Moreover, such a change might be expected to be accompanied by some abnormality at meiosis, such as pairing failures or alterations in chromosome condensation and coil formation, that could be visibly identified by a cytological examination of pollen mother cells.

Materials and Methods

Thirty six F_1 plants of *Hordeum Vulgare* were used from the cross, $\underline{f + +} / \underline{f + +}$ ♀ X $\underline{+ e\ tr} / \underline{+ e\ tr}$ ♂ (University of Alberta accession numbers 116 and 147 respectively). Three second chromosome marker genes: chlorina (*f*), elongated, awned outer glume (*e*), and triple-awn lemma (*tr*), linked in the order listed are subject to linkage study.

The plants were grown in a growth chamber at 23 ± 1°C. At the age of one month the plants showed a marked uniformity in appearance. After a complete leaching with tap water and a subsequent three-day water-free period, treatments were commenced by watering the plants with Hoagland's solution containing selenium in different concentrations. The selenium was added as $Na_2SeO_4 \cdot 7H_2O$. Its concentrations for the four treatment groups are shown in Table I-1. Ten treatments were given at two-day intervals, and involved the application of a 250 ml aliquot of solution to each pot. On the day after treatment the pots were irrigated with distilled water to prevent lethal accumulation of selenate. Treatments were terminated when the heads of first tillers approached one inch in length, at which stage meiosis was in progress. Fifteen days after the last treatment the F_1 plants were fertilized once with a nitrogen-free potassium-phosphorus fertilizer. Pollen mother cells were collected at meiosis for cytological study. The

TABLE I-1

The Concentration of Selenium in Treatments

Treatment Group	Number of Plants	Conc. of Se (in ppm)
1	9	0.00
2	9	0.50
3	9	1.60
4	9	5.00

aceto-carmine squash technique (Belling 1926) was used for the preparation of microslides. The F_2's were planted in the field and subsequently harvested and classified phenotypically.

Results of Genetic Analysis

The phenotypic classification of F_2 plants with respect to *f*, *e* and *tr* are shown in Table I-2. Columns 2, 3, 4 and 5 show the segregations of the three genes in F_1's grown under different selenium concentrations. It can be seen from the last two rows that *f* and *e* are very closely linked. Since detectable differences with treatment are not to be expected with such close linkage, the measurement of *f-e* linkage has been excluded from further analysis.

Table I-3 shows the dihybrid segregation frequencies for *e-tr* and *f-tr* obtained by reorganizing the data of Table I-2. A linkage-detection test was then applied to the recombination data for *e-tr* and *f-tr* for different selenium treatment concentrations. The results are shown in Table I-4. The $\underline{X}^2$ values under the columns designated "Overall linkage test" were estimated against the 9:3:3:1 ratio for independent segregation, and carry three degrees

of freedom. In all selenium treatments, both *e-tr* and *f-tr* show linkage. In the control, *e-tr* shows independent segregation, and the X^2 value for *f-tr*, although significant, is caused by an excess of recombinants rather than of parental types. *f* and *tr* must therefore be considered to segregate independently in these controls. (For confirmation of this point see Table I-5 for *y* value, for *f-tr* in Treatment Group 1).

The $\underline{X}^2$ value from each of the overall linkage tests was then partitioned into its components for further testing, the three degrees of freedom being apportioned as follows:

1 D.F. for the segregation *+* vs *e*; *+* vs *f*.

1 D.F. for the segregation *+* vs *tr*.

1 D.F. for the segregation *parental* vs *recombinant*.

The component X^2's are shown in their respective columns of Table I-4. The segregations for *+* vs *tr* in treatments 2 and 3, and that for *+* vs *f* in the control deviate significantly from a 3:1 ratio. These disturbed ratios have not been satisfactorily explained; the disturbances in the *+* vs *tr* segregations may have been caused by the occurrence of an unexpected second factor for triple awn lemma (see Kasha and Walker 1963), but this is extremely unlikely since all the crosses involved two true breeding lines.

In spite of this the 1 D.F. linkage detection test confirmed the results obtained from the overall linkage test. A further test for independence, in which the actual ratios for each gene were used to calculate the expected segregations (shown in the last column under each treatment group of Table I-4) again confirmed these latter results.

Even in the presence of disturbed single-gene ratios the product method, according to Mather (1951), gives a very accurate estimate

TABLE I-2

Phenotypic classification of F_2 plants

Phenotype of F_2 plants	Treatment Group			
	1 Se, 0.00 ppm	2 Se, 0.50 ppm	3 Se, 1.60 ppm	4 Se, 5.00 ppm
+ + +	344	346	420	334
+ + tr	112	235	160	89
+ e t	145	93	70	62
+ e tr	50	148	158	94
f + +	78	203	185	137
f + tr	53	78	60	31
f e +	2	0	0	0
f e tr	0	0	0	0
Total	784	1103	1053	747

TABLE I-3

Segregation of e-tr and f-tr in four treatment groups

Linkage Group	Phenotypic class	Treatment Group			
		1	2	3	4
e-tr	+ +	422	549	605	470
	e +	147	93	70	62
	+ tr	165	313	220	120
	e tr	50	148	158	95
f-tr	+ +	489	439	490	395
	f +	80	203	185	137
	+ tr	162	383	318	183
	f tr	53	78	60	32

TABLE I-4

X^2 test for linkage detection between _e_ and _tr_; _f_ and _tr_ (see text)

Linkage Group	Treatment Group									
	1					2				
	Overall linkage test X^2 (D.F.=3, 9:3:3:1)	Component analysis of overall linkage test X^2			Test for independence X^2 (D.F.=1)	Overall linkage test X^2 (D.F.=3, 9:3:3:1)	Component analysis of overall linkage test X^2			Test for independence X^2 (D.F.=1)
		Segregation for e or f (D.F.=1) 3:1	Segregation for tr (D.F.=1) 3:1	linkage (D.F.=1)			Segregation for e or f (D.F.=1) 3:1	Segregation for tr (D.F.=1) 3:1	linkage (D.F.=1)	
e-tr	3.04	-	-	-	-	215.94*	6.84	164.93*	44.20*	48.77*
f-tr	40.50*	27.00	2.40	11.10*	12.90*	204.38*	0.13	165.94*	38.31*	30.53*
	Treatment Group									
	3					4				
e-tr	213.04*	6.39	66.69*	139.96*	142.28*	102.25*	6.32	5.79	90.23*	97.60*
f-tr	92.59*	1.69	68.15*	22.75*	18.06*	19.36*	2.24	5.70	11.42*	10.33*

of the recombination fraction and was therefore used in this experiment. The formulas for the product method are:

$$\frac{m_2m_3}{m_1m_4} = \frac{(2y-y^2)^2}{(3-2y+y^2)\ (1-2y+y^2)} \qquad \text{(coupling phase)}$$

$$\frac{m_1m_4}{m_2m_3} = \frac{y^2(2 + y^2)}{(1 - y^2)^2} \qquad \text{(repulsion phase)}$$

Where $\underline{m}_1$ to $\underline{m}_4$ designate the observed frequencies of the four phenotypic classes, and $\underline{y}$ stands for the recombination fraction. The results of the calculations are tabulated in Table I-5.

TABLE I-5

Recombination fraction (y) values between e-tr and f-tr in selenium treatments

Treatment Group	Concentration of Selenium (ppm)	Linkage Group e-tr	 f-tr
1	0.00	0.5196±0.0273	0.55
2	0.50	0.3617±0.0188	0.3854±0.0253
3	1.60	0.2697±0.0165	0.4027±0.0255
4	5.00	0.2731±0.0197	0.4039±0.0302

From the e-tr column of Table I-5 it may be seen that the rate of crossing over in this segment varies inversely and consistently with the concentration of selenium in the treatment for all treatments, although the values in treatment groups 3 and 4 (0.2697 and 0.2731 respectively) are not significantly different. For f-tr the value of $\frac{m_1m_4}{m_2m_3}$ in the control is 1.9997, whereas the largest product value given in the table from Immer and Handerson (1943) for the calculation of percent recombination is 1.43 with a corresponding $\underline{y}$ value of 0.55. Similar excesses over the theoretical limit of 0.5 for $\underline{y}$ are discussed in Bailey (1961); they are considered to indicate map distances considerably in excess of the minimum required for

independence (viz. 0.5). Although the estimated values for y in the three selenium-treatment groups do not show a consistent decline with increasing treatment, a comparison of their means and standard deviations indicates that they differ significantly from the control. These results indicate that the crossing-over rate between f and tr is reduced in the selenium-treated groups but that in these the difference in selenium concentration did not give significant differences in y values.

It may be concluded from these results that both e-tr and f-tr show linkage in all selenium treatments, and independent segregation in the controls. The crossing over rate for the two higher concentrations was reduced to about half that of the control. It is apparent from the foregoing that selenium reduces crossing over.

Results of Cytological Study

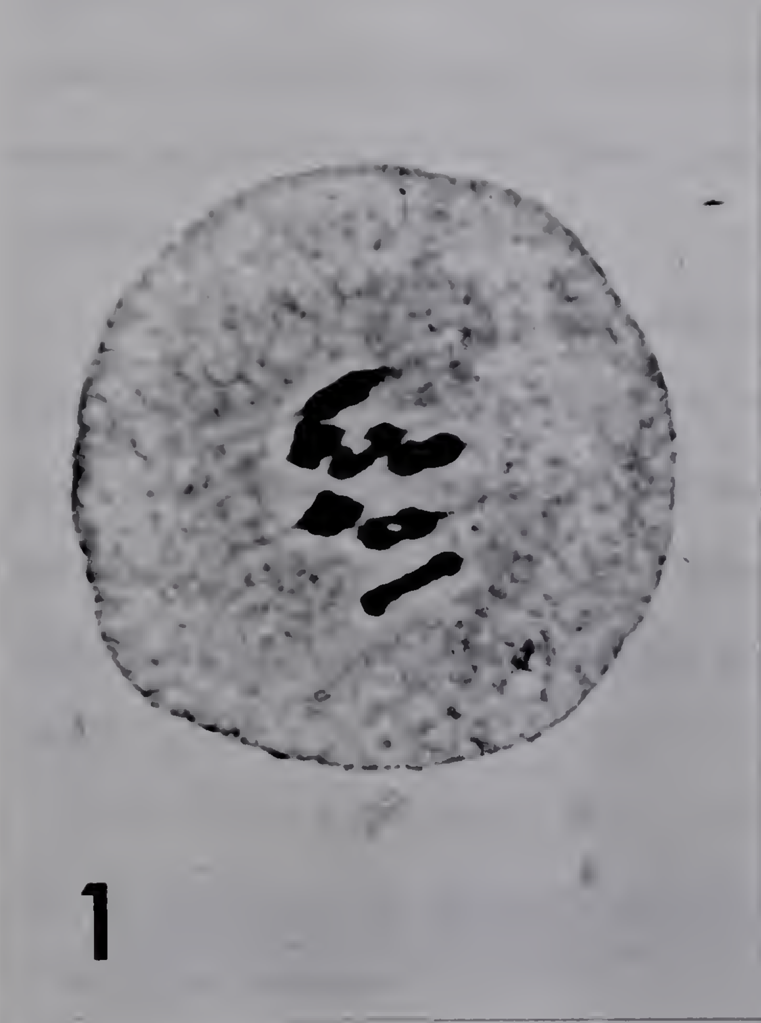
Typical results are depicted in Plate I-1. Figure 1 shows a representative normal cell at metaphase I obtained from the control group. The normal seven bivalent chromosomes are evident. Figures 2, 3 and 4 show representative cells of the highest concentration selenium treatment (5.00 ppm). The cells in figures 3 and 4 are at metaphase I; figure 2 shows the anaphase II stage. These preparations demonstrated several interesting phenomena.

1. Of approximately 350 pollen mother cells in the selenium treated groups examined for pairing abnormalities all showed the usual seven bivalents at pachytene.

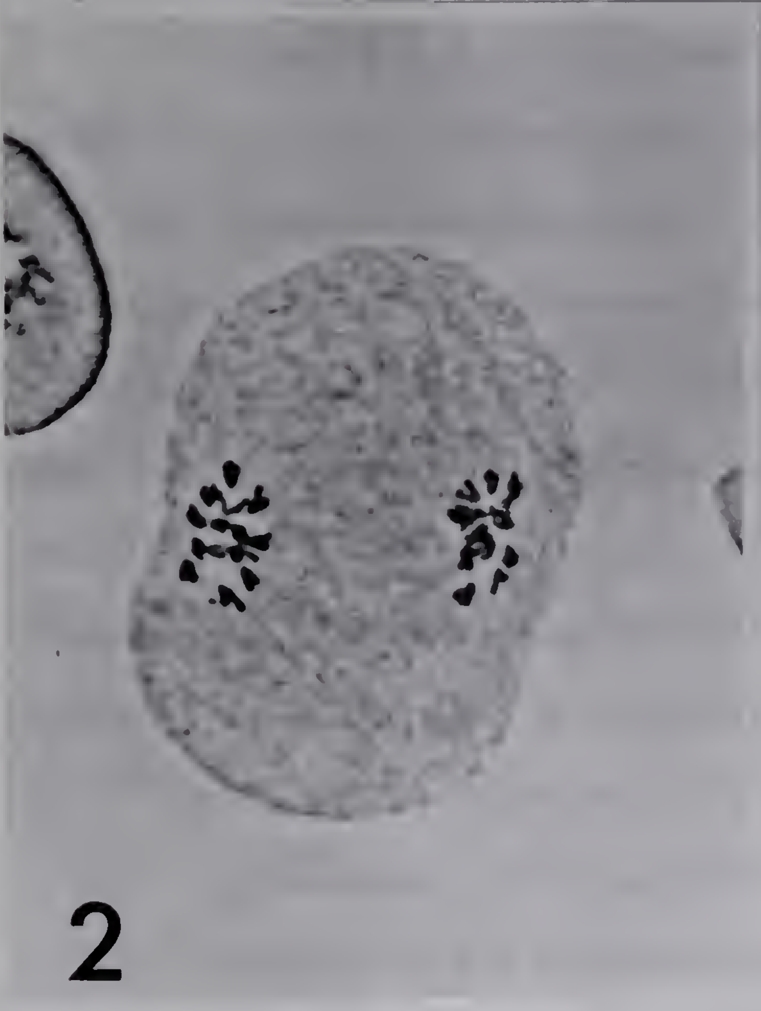
2. In preparations from the highest concentration (5.00 ppm) treatment group 157 pmc's were examined; four were observed to be polyploid. No such effect was noted in the other groups. This indicates

PLATE I-1

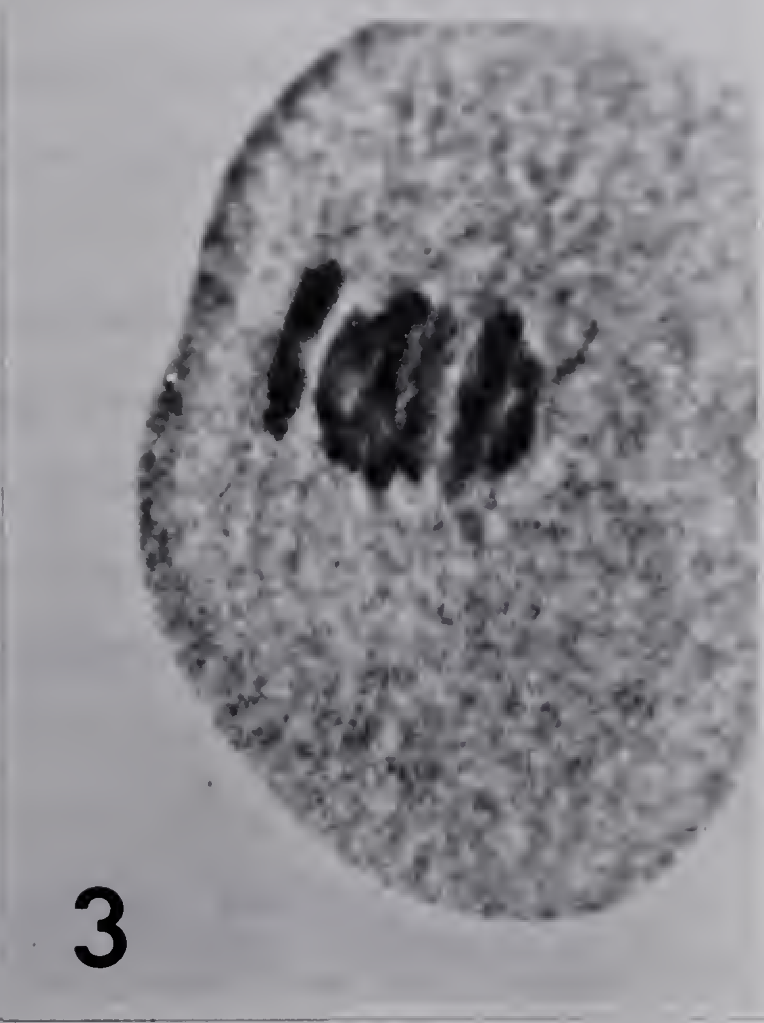
The effect of sodium selenate on the meiotic cells of barley.



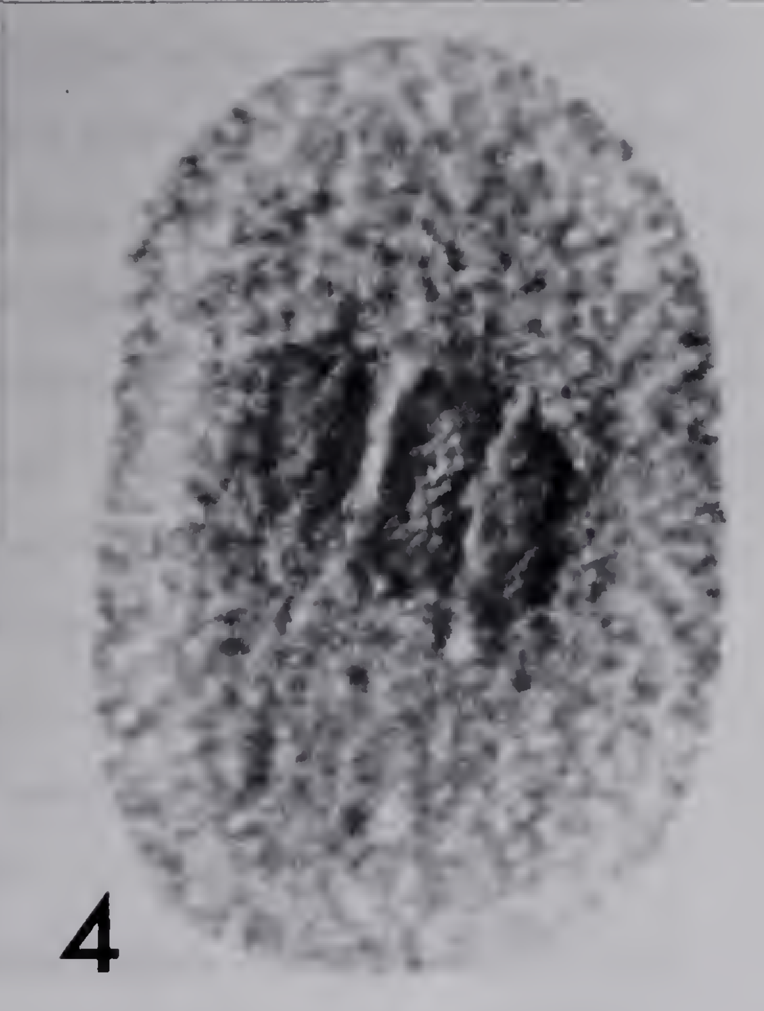
1



2



3



4

that the treatment probably destroyed or interfered with the spindle apparatus or its function at Meiosis I. It is therefore possible that selenium was incorporated into the spindle fibres, or into enzymes mediating their activity, leading to the failure of homologues to migrate poleward at anaphase I.

3. In preparations from the treatment groups of highest concentration, 68 of the preparations exhibited apparent stretching and dispersion of metaphase I chromosomes (Figs. 3 and 4). The degree of deformation varied over a wide range. These observations strongly suggests that selenium altered chromosomal condensation and coil formation in some way.

II. A Study of the Effects of Selenocystine, Selenomethionine and Glutathione on Crossing Over and Interference in Drosophila melanogaster.

Experimental results from the foregoing genetic and cytological studies of selenium treated barley have provided evidence that protein is involved in the meiotic mechanism leading to genetic recombination. Whether the protein concerned is enzymatic or structural remains to be determined. Although the selenium effect on chromosome morphology provides a suggestion that it may be predominantly structural, the possibility cannot be rejected that it might be enzymatic. The present study is designed to test these alternatives against a background of crossover reduction with selenium treatment according to the following criteria:

1. Whether or not there is a reduction in the coefficient of coincidence with treatment.
2. Whether or not magnitude differences in the intensity of crossover reduction exist between different regions of the chromosome.

The theoretical basis for these criteria rests on the consideration that an enzyme-mediated breakage event may be expected to be distributed entirely at random along the chromosome strands so that the probability of a crossover at any site should be related entirely to the concentration or the activity of enzyme macromolecules; and, in contrast, on the assumption that a stress-mediated breakage event depends on the distribution of stress-inducing forces along the chromosome strand.

If the protein affected is enzymatic, selenium treatment should reduce the rate of crossing over in adjacent segments but the coincidence coefficient with respect to the two segments should not be changed. For if the inherent properties in two adjacent segments of a strand impose a restriction on coincidence by a restriction on the proximity of acceptor sites for an enzyme solely responsible for crossing over, this restriction should be constant regardless of the concentration of the effective enzyme. As a corollary to this, it may be added that any change observed in this coefficient should be the result of a change in the properties of the chromosomal (nucleoprotein) strand.

On the other hand, if the protein affected is chromosomal, a change (reduction) in the coefficient of coincidence may be expected because of a mutual interplay of the crossover-reductions of adjacent segments. A mathematical illustration of these concepts follows.

Let r_1 and r_2 be the fractional reductions in crossing over frequency with treatment for segments 1 and 2. Recombination fractions are designated as y_1, y_2, y_{12} and y_1^s, y_2^s, y_{12}^s; the superscripts referring to controls and treatment respectively, the subscripts referring to each segment and the two segments combined.

The effect of treatment on the double crossover component may then be expressed in terms of r_1 and r_2 as follows:

frequency of coincident loss of crossovers in the two segments: $r_1 r_2$ (1)

frequency of loss of crossovers in segment 1 only: $r_1(1 - r_2)$ (2)

frequency of loss of crossovers in segment 2 only: $r_2(1 - r_1)$ (3)

frequency of unaffected double crossovers: $(1 - r_1)(1 - r_2)$ (4)

With the hypothesis that crossing over is an enzyme-mediated process:

$$y_1{}^s = y_1(1 - r_1) \tag{5}$$

$$y_2{}^s = y_2(1 - r_2) \tag{6}$$

and since $(1 - r_1)(1 - r_2)$ represents the reduced frequency of double-crossovers with treatment,

$$y_{12}{}^s = y_{12}(1 - r_1)(1 - r_2) \tag{7}$$

Hence the coefficient of coincidence with treatment will be,

$$C^s = \frac{y_{12}{}^s}{y_1{}^s y_2{}^s} = \frac{y_{12}(1 - r_1)(1 - r_2)}{y_1(1 - r_1) y_2(1 - r_2)} = \frac{y_{12}}{y_1 y_2} = C \tag{8}$$

On the other hand, with the hypothesis that mechanical forces for a torsional nature mediate crossing over, since any elimination in a segment of double crossovers by treatment, would not be expected to involve a significant loss of single crossovers in adjacent subsegments, it may be expected that the C value $\frac{(y_{12})}{(y_1 y_2)}$ would be materially reduced. Consequently the study of interference in a selenium treatment experiment should provide a means of determining whether the protein involved is enzymatic or chromosomal.

The second theoretical criterion for distinguishing between the effect of enzymatic and chromosomal proteins on crossing over resides in the patterns they may be expected to exhibit in reducing recombination rates. With control of the process entirely by enzymes an equal intensity of

reduction of crossing over is expected in each segment of a chromosome arm marked off by multiple marker genes. The extent of the effect should be identical from one segment to another, at least if the sites for exchange are randomly distributed along the chromosome. In contrast, magnitude differences in the intensity of reduction of crossing over in different regions of the chromosome arm (as for example, with a great reduction in the middle of a chromosome arm and a gradually decreasing reduction towards its distal end), would indicate the presence of a varying mechanical force along the chromosome or the presence of structural differences along the chromosome related in some way to crossing over.

In addition to selenium treatments the following study included an investigation into the effects of glutathione on crossing over. If disulfide bonding phenomena are involved in the process of crossing over, this substance might be expected to affect the formation and dissolution of these bonds, and hence to affect crossing over, due to its own sulfur content. Its effects might thus be comparable with those of selenium.

Material and Methods

Drosophila melanogaster was used in this experiment. In all cases crossing over was measured in the X chromosome using the following markers (map distances as listed in Bridges and Brehme 1944): *y* = yellow, 0.0; *cv* = crossveinless, 13.7; *v* = vermilion, 33.0; *f* = forked 56.7. The interval between *y* and *cv* is designated segment 1; *cv* and *v*, segment 2; *v* and *f* segment 3. The region from *y* to *f* includes most of the euchromatic region of the X chromosome. This region was chosen with the purpose of avoiding the genetic effects of heterochromatin (Suzuki 1964).

Four-point test crosses were performed in all experiments.

Females heterozygous with respect to the four genes were obtained by crossing y cv v f/y cv v f ♀ with + + + +/↑♂ (the former stock generously provided by the Department of Genetics, University of Wisconsin, the latter by the Department of Biology, Yale University) and backcrossing the progeny to mutant males from the female parent strain. Both parental stocks had high viability.

The expectation that selenium might substitute for sulphur in amino acids, suggested in demonstrations that it was incorporated into plant proteins and confirmed by the isolation and later the synthesis of seleno-cystine and selenomethionine, provided a working hypothesis for a mechanism of seleno-sulphur substitution that would permit the present test. At the time of the earlier tests, no organic selenium compounds had been isolated, and therefore barley, which in common with some other cereals is known to be capable of assimilating considerable amounts of inorganic selenium, was chosen as a test organism. The test proved unsuitable for tests of interference metrics, and, with the advent of seleno-amino acids in purified form, which permits the introduction of selenium into the food in a utilizable form, Drosophila was chosen as a more suitable test organism.

Selenocysteine is at present not available, therefore selenocystine was used for treatment, since it is known that yeast cells, an important food source for larvae, contain an enzyme (cystine reductase) highly specific for the reduction of cystine to cysteine (Nickerson and Romano 1952, 1954) thus:

$$\text{Cystine} + \text{DPNH} \xrightarrow{\text{cystine reductase}} 2\ \text{cysteine} + \text{DPN}^{+}$$

Since selenocystine is insoluble in neutral solvent, it was prepared in acetic-acetate buffer at a pH of 4.7 for treatment. The culture medium has a pH of 5.0, at which no selenocystine precipitation is expected.

Methionine is an essential amino acid for most animals and is

related to cysteine metabolism. Selenomethionine was therefore used for treatment for comparison with selenocystine. It was prepared in an identical manner.

Glutathione, studied in the third group of treatments, is a known constituent of yeast, present in reasonably large amounts. The amount and form (reduced vs oxidized) may be expected to vary during culture. To provide for a graded series of concentrations, without resorting to an extensive chemical analysis, two treatment groups were included, the one being deprived of yeast (medium infected with one fifth the normal amount of yeast solution) and the other being provided with 1000 ppm aqueous solution of glutathione (SH) in addition to the normal complement of yeast.

The treatment groups with respect to the nature and concentrations of chemicals are tabulated in Table II-1.

TABLE II-1

Treatment Chemicals and Concentrations

Chemical treatments	Concentrations (ppm)							
selenocystine	0.00	0.50	1.60	5.00	25.00	100.00		
selenomethionine	0.00	0.50	1.60	5.00	-	-		
glutathione (SH)	0.00	-	-	-	-	-	-yst 4/5*	1000.00

* yeast deprived treatment (see text)

The medium into which the parental flies ovulated contained the treatment chemicals. When the F_1 adults emerged from this medium, four virgin females not older than 24 hours were mated with four homozygous recessive males, in quarter pint bottles of medium lacking treatment

chemicals, at 25 ± 1°C. This procedure was repeated at three day intervals to give four broods of testcross progeny flies for linkage analysis. The corn meal culture medium was formulated according to the recipe of the Biology Division, Oak Ridge National Laboratories, Oak Ridge, Tennessee.

The F_1 larvae received treatment from the earliest developmental stages up to the last instar, but received no treatment from the pupal stage on. F_1 females were transferred prior to maturity to selenium-free medium along with tester males for the production of testcross progeny. Since in Drosophila melanogaster it is well known that the initial progeny are descended from meiocytes which were undergoing meiosis during the mother's larval stage, the more marked effects of treatment would be expected in the earlier broods.

Observations and Results

The observation that selenium is toxic to most organisms and causes considerable lethality even at low concentrations (Trelease, 1949) is confirmed by these treatments, although the toxic effects appeared only at relatively high concentrations of the seleno-amino acids.

In the 100 ppm selenocystine treatment only twenty pupae were obtained from six bottles; most of the larvae died prior to pupation. Among the twenty pupae, three males and one female hatched. These individuals required twice as much time as did normal (untreated) ones to reach maturity, and attained only about half the normal size. The larvae in this treatment were small, slender and listless; they gradually darkened and at death were black. These observations demonstrate that high levels of selenocystine probably cause a serious physiological disturbance ending in death. This disturbance might be attributed to inactivation of meta-

bolically active proteins.

Except for a somewhat lower yield of adults with the 25 ppm treatment, no evidence of an appreciable toxic effect was found in either the rest of the selenocystine or in the methionine treatments.

Among the testcross offspring, again no evidence of differences in progeny size either between treatment groups or broods was found. A relatively low yield of the 0.5 ppm selenomethionine group resulted from bacterial contamination of the medium. Furthermore, no differential viability was observed between genotypes.

In the yeast-deprived treatment a relatively low yield of flies was obtained. It is not known if this loss of yield resulted from the yeast deprivation or from other causes. There was no difference in the 1000 ppm glutathione treatment with respect to the yield, nor was differential viability between genotypes in these treatments observed.

The crossover-scoring data for all treatments are tabulated in Table II-2. The control is replicated and appears in the first row of each of the three chemical (selenocystine, selenomethionine and glutathione) treatments. Two interference indices were calculated for this experiment: the coincidence coefficient $\underline{C}$, and the Kosambi coefficient $\underline{K}$. According to Bailey (1961) the $\underline{C}$ value suffers from the disadvantage of depending on the recombination fractions $\underline{y}_1$ and $\underline{y}_2$ of the two intervals from which it is calculated. The Kosambi coefficient is an adjusted index, that theoretically does not vary with variations in $\underline{y}_1$ and $\underline{y}_2$ and hence may be considered an index of the level of interference in that region for which it is calculated.

The recombination fraction, $\underline{y}$, and map distance, $\underline{x}$, are calculated according to the Kosambi model. The formulae and their applications to the calculations of the estimates $\underline{y}$, $\underline{x}$, $\underline{K}$ and $\underline{C}$ are found in Bailey (1961) and

TABLE II-2a

Observed frequencies of the progeny of backcrosses:
y cv v f/++++♀ X y cv v f/↾♂

Treatment	Concentration (ppm)	Progeny from Backcross								
		1st Brood								
		Modes of Gamete Formation								
		0	1	2	3	1.2	1.3	2.3	1,2,3	Totals
Seleno-cystine	0.00	235	52	99	85	6	20	24	0	521
	0.50	226	66	89	88	10	25	26	1	531
	1.60	514	134	209	158	11	47	39	2	1114
	5.00	229	57	108	90	2	11	11	1	509
	25.00	281	50	105	97	2	21	11	0	567
Seleno-methionine	0.00	235	52	99	85	6	20	24	0	521
	0.50	118	38	64	48	3	14	12	0	297
	1.60	303	83	139	99	10	31	23	0	688
	5.00	301	78	148	121	8	26	31	1	714
Gluta-thione (SH)	0.00	235	52	99	85	6	20	24	0	521
	-yst (4/5)	198	47	89	85	4	13	8	0	444
	1000.00	289	67	121	96	2	19	21	0	615

Modes of gamete formation:

0 = no recombination
1, 2, 3 = recombination in region 1, 2, 3 only
1, 2; 1, 3; 2, 3; = simultaneous recombination in regions 1 and 2; 1 and 3, 2 and 3.
1, 2, 3 = simultaneous recombination in regions 1, 2 and 3.

TABLE II-2b

Observed frequencies of the progeny of backcrosses:
y cv v f/++++♀ X y cv v f/↑♂

Treatment	Concentration (ppm)	Progeny from Backcross								
		2nd Brood								
		Modes of Gamete Formation								
		0	1	2	3	1.2	1.3	2.3	1,2,3	Totals
Seleno-cystine	0.00	250	60	120	81	7	20	20	0	558
	0.50	284	92	116	97	2	30	26	2	649
	1.60	327	91	171	110	11	25	22	1	758
	5.00	218	54	84	70	9	17	21	3	476
	25.00	331	69	130	93	7	22	24	1	677
Seleno-methionine	0.00	250	60	120	81	7	20	20	0	558
	0.50	116	40	55	43	1	17	9	0	281
	1.60	200	68	99	67	3	16	24	0	477
	5.00	197	73	83	85	8	20	14	0	480
Gluta-thione (SH)	0.00	250	60	120	81	7	20	20	0	558
	-yst (4/5)	295	79	121	83	6	14	19	0	617
	1000.00	369	102	153	137	4	37	28	0	830

Modes of gamete formation:
0 = no recombination
1, 2, 3 = recombination in region 1, 2, 3 only
1, 2; 1, 3; 2, 3; = simultaneous recombination in regions 1 and 2; 1 and 3, 2 and 3.
1, 2, 3 = simultaneous recombination in regions 1, 2 and 3.

TABLE II-2c

Observed frequencies of the progeny of backcrosses:
y cv v f/++++♀ X y cv v f/↑♂

Treatment	Concen-tration (ppm)	Progeny from Backcross								
		3rd Brood								
		Modes of Gamete Formation								
		0	1	2	3	1.2	1.3	2.3	1,2,3	Totals
Seleno-cystine	0.00	247	81	121	100	5	29	27	2	612
	0.50	126	52	61	40	2	12	12	0	305
	1.60	136	49	59	55	6	13	15	1	334
	5.00	108	38	47	27	6	12	5	0	243
	25.00	255	58	118	81	3	12	19	0	546
Seleno-methionine	0.00	247	81	121	100	5	29	27	2	612
	0.50	75	24	39	23	2	12	5	0	180
	1.60	214	73	91	73	6	20	13	1	491
	5.00	185	59	85	77	7	19	12	1	445
Gluta-thione (SH)	0.00	247	81	121	100	5	29	27	2	612
	-yst (4/5)	284	86	112	100	4	20	18	0	624
	1000.00	283	83	128	117	6	20	28	0	665

Modes of gamete formation:
0 = no recombination
1, 2, 3 = recombination in region 1, 2, 3 only
1, 2; 1, 3; 2, 3; = simultaneous recombination in regions 1 and 2; 1 and 3, 2 and 3.
1, 2, 3 = simultaneous recombination in regions 1, 2 and 3.

TABLE II-2d

Observed frequencies of the progeny of backcrosses:
y cv v f/++++♀ X y cv v f/↑ ♂

Treatment	Concen-tration (ppm)	Progeny from Backcross								
		4th Brood								
		Modes of Gamete Formation								
		0	1	2	3	1.2	1.3	2.3	1,2,3	Totals
Seleno-cystine	0.00	210	66	98	77	5	14	12	1	483
	0.50	180	57	88	79	6	24	20	2	456
	1.60	343	97	165	101	18	37	25	2	788
	5.00	162	54	70	51	7	23	13	0	380
	25.00	216	57	97	84	2	19	11	0	486
Seleno-methionine	0.00	210	66	98	77	5	14	12	1	483
	0.50	65	26	21	25	5	10	7	1	160
	1.60	212	73	108	72	9	25	26	0	525
	5.00	174	52	76	62	9	13	15	1	402
Gluta-thione (SH)	0.00	210	66	98	77	5	14	12	1	483
	-yst (4/5)	198	64	83	74	6	23	12	2	462
	1000.00	253	75	96	86	5	23	19	2	559

Modes of gamete formation:
0 = no recombination
1, 2, 3 = recombination in region 1, 2, 3 only
1, 2; 1, 3; 2, 3; = simultaneous recombination in regions 1 and 2, 1 and 3, 2 and 3.
1, 2, 3 = simultaneous recombination in regions 1, 2 and 3.

TABLE II-3a

Observed frequencies of 1st brood progeny reorganized from Table II-2. The four-gene interval is divided into two threepoint segments with segment 2 (cv-v) overlapping

Treatment	Concentration (ppm)	y $\overset{1}{-}$ cv $\overset{2}{-}$ v					cv $\overset{2}{-}$ v $\overset{3}{-}$ f				
		Modes of Gamete Formation					Modes of Gamete Formation				
		0	1	2	1,2	Totals	0	2	3	2,3	Totals
Seleno-cystine	0.00	320	72	123	6	521	287	105	105	24	521
	0.50	314	91	115	11	531	292	99	113	27	531
	1.60	672	181	248	13	1114	648	220	205	41	1114
	5.00	319	68	119	3	509	286	110	101	12	509
	25.00	378	71	116	2	567	331	107	118	11	567
Seleno-methionine	0.00	320	72	123	6	521	287	105	105	24	521
	0.50	166	52	76	3	297	156	67	62	12	297
	1.60	402	114	162	10	688	386	149	130	23	688
	5.00	422	104	179	9	714	379	156	147	32	714
Gluta-thione (SH)	0.00	320	72	123	6	521	287	105	105	24	521
	-yst (4/5)	283	60	97	4	444	245	93	98	8	444
	1000.00	385	86	142	2	615	356	123	115	21	615

TABLE II-3b

Observed frequencies of 2nd brood progeny reorganized as in Table II-3a

Treatment	Concentration (ppm)	y $\underline{1}$ cv $\underline{2}$ v					cv $\underline{2}$ v $\underline{3}$ f				
		Modes of Gamete Formation					Modes of Gamete Formation				
		0	1	2	1.2	Totals	0	2	3	2.3	Totals
Seleno-cystine	0.00	331	80	140	7	558	310	127	101	20	558
	0.50	381	122	142	4	649	376	118	127	28	649
	1.60	437	116	193	12	758	418	182	135	23	758
	5.00	288	71	105	12	476	272	93	87	24	476
	25.00	424	91	154	8	677	400	137	115	25	677
Seleno-methionine	0.00	331	80	140	7	558	310	127	101	20	558
	0.50	159	57	64	1	281	156	56	60	9	281
	1.60	267	84	123	3	477	268	102	83	24	477
	5.00	282	93	97	8	240	270	91	105	14	480
Gluta-thione (SH)	0.00	331	80	140	7	558	310	127	101	20	558
	-yst (4/5)	378	93	140	6	617	374	127	97	19	617
	1000.00	506	139	181	4	830	471	157	174	28	830

TABLE II-3c

Observed frequencies of 3rd brood progeny reorganized as in Table II-3a

Treatment	Concentration (ppm)	y $\underline{1}$ cv $\underline{2}$ v					cv $\underline{2}$ v $\underline{3}$ f				
		Modes of Gamete Formation					Modes of Gamete Formation				
		0	1	2	1.2	Totals	0	2	3	2.3	Totals
Seleno-cystine	0.00	347	110	148	7	612	328	126	129	29	612
	0.50	166	64	73	2	305	178	63	52	12	305
	1.60	191	62	74	7	334	185	65	68	16	334
	5.00	135	50	52	6	243	146	53	39	5	243
	25.00	336	70	137	3	546	313	121	93	19	546
Seleno-methionine	0.00	347	110	148	7	612	328	126	129	29	612
	0.50	98	36	44	2	180	99	41	35	5	180
	1.60	287	93	104	7	491	287	97	93	14	491
	5.00	262	78	97	8	445	244	92	96	13	445
Gluta-thione (SH)	0.00	347	110	148	7	612	328	126	129	29	612
	-yst (4/5)	384	106	130	4	624	370	116	120	18	624
	1000.00	400	103	156	6	665	366	134	137	28	665

TABLE II-3d

Observed frequencies of 4th brood progeny reorganized as in Table II-3a

Treatment	Concentration (ppm)	y $\underline{1}$ cv $\underline{2}$ v					cv $\underline{2}$ v $\underline{3}$ f				
		Modes of Gamete Formation					Modes of Gamete Formation				
		0	1	2	1.2	Totals	0	2	3	2.3	Totals
Seleno-cystine	0.00	287	80	110	6	483	276	103	91	13	483
	0.50	259	81	108	8	456	237	94	103	22	456
	1.60	444	134	190	20	788	440	183	138	27	788
	5.00	213	77	83	7	380	216	77	74	13	380
	25.00	300	76	108	2	486	273	99	103	11	486
Seleno-methionine	0.00	287	80	110	6	483	276	103	91	13	483
	0.50	90	36	28	6	160	91	26	35	8	160
	1.60	284	98	134	9	525	285	117	97	26	525
	5.00	236	65	91	10	402	226	85	75	16	402
Gluta-thione (SH)	0.00	287	80	110	6	483	276	103	91	13	483
	-yst (4/5)	272	87	95	8	462	262	89	97	14	462
	1000.00	339	98	115	7	559	328	101	109	21	559

Wallace (1957). The best estimate of K from four-point data, according to Owen (ref. Bailey 1961), is provided by the separate calculation of the maximum likelihood estimates of K for the two three-gene intervals (triads). This method is followed here. The original data (Table II-2) are thus reorganized in accordance with the requirement of two separate triads (y-cv-v, cv-v-f) as shown in Table II-3. The four parts (a, b, c and d) of Table II-3 represent the four broods of offspring.

The estimated recombination fraction y of each segment for each treatment and the corresponding map distance is shown in Tables II-4 and II-5 respectively. It may be noted that, since the triads overlap in region 2 (cv-v), and the y and x values of the two triads are calculated independently, there are, therefore, two estimates for each of these values in the mid-region. However, in no case did these two estimates for a region deviate significantly. The Kosambi and coincidence coefficients for the two triads are shown in Tables II-6 and II-7. Elementary interference theory does not invoke map distances, and Kosambi values, having a rather specialized use in the comparison of levels of interference in different regions, are not useful in the test described earlier (page 28) for enzymic vs structural influence over crossing over.

With respect to the effect on recombination fractions, there is a consistent pattern in both first and second broods (Fig. II-1a and 1b) with selenocystine treatment. The curves are somewhat erratic in the 3rd and 4th broods. However, the smoothed curves (not illustrated) for broods 3 and 4 actually follow the same pattern as those of 1 and 2.

In the first brood from selenocystine treatment (Fig. II-1a) a consistent decrease of y value is evident with increase of chemical dosage

in all three regions (y-cv, cv-v, and v-f) the two exceptions being in the y-cv region at lower concentrations (0.50 and 1.60 ppm). The decrease in cv-v becomes significant at 25.00 ppm.

At the same concentration the decrease of y for the control in the middle segment (cv-v) is larger than it is in the outer segments (viz. 2.04 for y-cv and 2.36 for v-f respectively) (see Table II-4). According to the second criteron proposed previously this result favours the hypothesis of stress-induced breakage.

The effect on y-cv at 0.50 and 1.60 ppm is especially interesting. An unexpected increase in recombination is shown; and the concentration producing the greatest increase appears to increase with successive broods (see Fig. II-1b, c, d). This unexpected increase, occurring at lower concentrations, may be due to local weaknesses at sites of incorporation of selenium into the chromosomal protein where these sites are widely separated. These weak sites would be susceptible to breakage and hence crossing over, but probably would not materially decrease the stress on the nucleoprotein structure. The peak at 0.5 ppm in the first brood shifts to higher concentrations (1.60, 5.00 ppm) in the later broods, suggesting that the accumulated selenium in the heterozygous females decreases with increasing age during the egg-laying period.

The erratic decrease in recombination of cv-v at 0.5 ppm in the second brood (Fig. II-1b) and that of v-f at 0.50 and 1.60 ppm in brood 3 (Fig. II-1c), as well as the increase in v-f at 0.50 ppm in the fourth brood (Fig. II-1d) may be related to a possible interaction between the (seleno)cystine metabolism of the yeast and that of the *Drosophila* larvae.

The effect, if any, of selenomethionine on recombination is very slight. However, two points of particular interest should be noted. Firstly,

there is a parallel relationship, with regard to the rank-pattern for y of the respective segments, between selenomethionine and selenocystine in broods 2, 3 and 4 (compare Figs. II-1b, c, d, with Figs. II-2b, c, d). In the second brood particularly, the graphs for the two chemical treatments are almost identical. That such similarities exist between the two chemicals in the later broods but not in the first one suggest that the yeast in the medium may possibly convert selenomethionine to selenocystine and that this conversion may not have reached appreciable proportions until after the first brood had been laid.

The coefficients of coincidence of both triads y-cv-v, cv-v-f in the first brood of the selenocystine treatment (Fig. II-4a) show a consistent decrease with increase of treatment concentration, although a small but insignificant increase has been found at the low (0.50 ppm) concentration. As with the recombination value of the y-cv region, a shifting of the peak to higher concentrations in the later broods is observed (see Fig. II-4b, c, d, and compare with Fig. II-1a, b, c, d). This slight increase in C value at low concentrations and its shifting to high concentrations in the later broods might also result from a formation of localized weak sites that do not affect an overall level of stress on the chromosome.

The decrease in C value for the cv-v-f segment becomes significant at the 5.00 ppm and 25.00 ppm levels to the extent that the standard errors at these levels do not overlap with those of the control, as shown in Fig. II-4a. As seen in the same figure the C value of y-cv-v at 25.00 ppm shows a decrease close to significance.

It may be recalled that, according to the first criterion for deciding between enzymes and chromosome-structural effects as a cause of breakage, a reduction in the coefficient of coincidence with a reduction in

crossing over favours the latter mechanism. The decreases just described, occurring in the presence of decreases in recombination (Fig. II-1a) strongly suggest that chromosomal stress is responsible for breakage.

In the selenomethionine treatment the graphs of the later broods (Figs. II-5b, c, d) seem to be comparable with their respective ones in the selenocystine treatment (Figs. II-4b, c, d) although the first brood (Fig. II-5a) does not. This observation supports the suggestion that there is an initial small conversion of selenomethionine to selenocystine by yeast. The selenocystine ingested would thus have its major effect in later broods.

A corollary to the relation between the increase of $\underline{y}$ and $\underline{C}$ values and the brood number is that the relative value of the Kosambi coefficient of the distal triad to that of the middle one for each brood shows a parallel pattern of effect. The calculated $K_{\underline{y\text{-}cv\text{-}v}}/K_{\underline{cv\text{-}v\text{-}f}}$ values in the selenocystine treatment are presented in Table II-8. The graphs of these values against brood number are plotted in Fig. II-7. A shift in the peak is evident with increasing brood number. These results provide further support for the 'localized weak site' hypothesis discussed above.

It is not clear how glutathione might act. Generally there is a lack of any consistent effect on $\underline{y}$ in any segment with either high concentration or deprivation of glutathione (eight out of twelve estimates are lower, but only slightly so; see Fig. II-3a, b, c, d). No consistent pattern of a differential segmental effect like that with selenocystine can be followed.

A fairly appreciable depression of $\underline{C}$ for both treatment and deprivation (except in brood IV, and in brood II segment cv-v) is observed (Fig. II-6a, b, c, d). Segment cv-v-f gives anomalous results, but y-cv-v in the first two broods (Fig. II-6a and b) shows consistent effects. If

valid, they would suggest an optimum concentration for low interference. The author has concluded, however, that no further information is gleaned from this study.

TABLE II-4

Recombination fractions

Treatment Group	Concentration (ppm)	Recombination Fraction (y)											
		Brood											
		1st			2nd			3rd			4th		
		Chromosomal Segment			Chromosomal Segment			Chromosomal Segment			Chromosomal Segment		
		y-cv	cv-v	v-f	y-cv	cv-v	v-f	y-cv	cv-v	v-f	y-cv	cv-v	v-f
Seleno-cystine	0.00	13.77 ±1.56	23.49 ±1.87 23.93 ±1.88	23.93 ±1.88	14.70 ±1.53	25.38 ±1.84 25.75 ±1.85	20.81 ±1.73	18.48 ±1.58	24.22 ±1.74 24.86 ±1.74	24.86 ±1.75	16.61 ±1.73	23.41 ±1.92 23.66 ±1.93	20.71 ±1.86
	0.50	18.72 ±1.70	22.64 ±1.83 23.03 ±1.83	26.02 ±1.87	18.40 ±1.54	21.27 ±1.62 21.95 ±1.63	22.94 ±1.66	20.20 ±2.34	23.04 ±2.44 24.01 ±2.44	20.01 ±2.31	18.58 ±1.84	24.38 ±2.02 24.79 ±2.02	26.76 ±2.07
	1.60	16.63 ±1.13	22.44 ±1.25 23.07 ±1.27	21.96 ±1.23	15.68 ±1.35	26.36 ±1.60 26.58 ±1.60	19.77 ±1.47	19.62 ±2.20	23.52 ±2.32 23.99 ±2.33	24.99 ±2.35	18.71 ±1.40	25.54 ±1.56 25.72 ±1.56	19.81 ±1.44
	5.00	12.69 ±1.53	22.38 ±1.87 22.54 ±1.88	21.59 ±1.83	16.88 ±1.73	23.82 ±1.95 24.15 ±1.96	23.14 ±1.92	22.56 ±2.68	22.54 ±2.71 22.67 ±2.71	17.77 ±2.45	21.47 ±2.11	22.43 ±2.16 22.81 ±2.16	21.82 ±2.14
	25.00	11.73 ±1.40	19.52 ±1.69 19.62 ±1.69	21.57 ±1.74	13.77 ±1.35	22.56 ±1.62 23.00 ±1.62	20.00 ±1.54	12.69 ±1.45	24.28 ±1.85 24.84 ±1.85	19.89 ±1.72	15.55 ±1.65	21.31 ±1.88 21.56 ±1.88	22.53 ±1.91

TABLE II-4 contd.

Treatment Group	Concentration (ppm)	Recombination Fraction (y)											
		Brood											
		1st			2nd			3rd			4th		
		Chromosomal Segment			Chromosomal Segment			Chromosomal Segment			Chromosomal Segment		
		y-cv	cv-v	v-f	y-cv	cv-v	v-f	y-cv	cv-v	v-f	y-cv	cv-v	v-f
Seleno-methionine	0.00	13.77 ±1.56	23.49 ±1.87 23.93 ±1.88	23.93 ±1.88	14.70 ±1.53	25.38 ±1.84 25.75 ±1.85	20.81 ±1.73	18.48 ±1.58	24.22 ±1.74 24.86 ±1.74	24.86 ±1.75	16.61 ±1.73	23.41 ±1.92 23.66 ±1.93	20.71 ±1.86
	0.50	17.47 ±2.24	25.10 ±2.54 25.65 ±2.54	23.69 ±2.49	19.23 ±2.39	22.10 ±2.49 22.71 ±2.50	23.68 ±2.54	19.36 ±2.95	24.22 ±3.22 24.54 ±3.22	21.63 ±3.08	25.83 ±3.44	20.87 ±3.21 21.11 ±3.23	26.16 ±3.47
	1.60	17.62 ±1.46	24.40 ±1.63 24.74 ±1.64	21.78 ±1.57	17.42 ±1.76	25.00 ±2.00 26.00 ±2.00	22.08 ±1.90	19.53 ±1.80	21.45 ±1.87 21.78 ±1.87	20.79 ±1.85	19.48 ±1.75	26.18 ±1.92 26.94 ±1.92	22.95 ±1.84
	5.00	14.68 ±1.36	25.36 ±1.63 25.77 ±1.63	24.79 ±1.61	20.57 ±1.84	20.54 ±1.87 20.71 ±1.87	23.65 ±1.95	18.65 ±1.86	22.53 ±1.99 22.60 ±1.99	23.59 ±2.02	17.79 ±1.93	24.69 ±2.14 24.88 ±2.14	21.90 ±2.07

TABLE II-4 contd.

Treatment Group	Concentration (ppm)	Recombination Fraction (y)											
		Brood											
		1st			2nd			3rd			4th		
		Chromosomal Segment			Chromosomal Segment			Chromosomal Segment			Chromosomal Segment		
		y-cv	cv-v	v-f	y-cv	cv-v	v-f	y-cv	cv-v	v-f	y-cv	cv-v	v-f
Glutathione	0.00	13.77 ±1.56	23.49 ±1.87 23.93 ±1.88	23.93 ±1.88	14.70 ±1.53	25.38 ±1.84 25.75 ±1.85	20.81 ±1.73	18.48 ±1.58	24.22 ±1.74 24.86 ±1.74	24.86 ±1.75	16.61 ±1.73	23.41 ±1.92 23.66 ±1.93	20.71 ±1.86
	-yst (4/5)	13.74 ±1.66	21.54 ±1.97 21.42 ±1.97	22.37 ±2.01	15.65 ±1.47	22.43 ±1.69 22.96 ±1.69	17.97 ±1.56	16.53 ±1.51	20.40 ±1.63 20.85 ±1.63	21.83 ±1.65	19.60 ±1.86	21.55 ±1.92 21.74 ±1.92	23.71 ±1.97
	1000.00	13.63 ±1.40	22.30 ±1.69 22.87 ±1.69	21.88 ±1.66	16.51 ±1.30	21.31 ±1.43 21.81 ±1.43	23.79 ±1.48	15.61 ±1.43	23.34 ±1.65 23.86 ±1.65	23.85 ±1.66	17.61 ±1.64	20.52 ±1.73 20.99 ±1.73	22.99 ±1.77

TABLE II-5

Map Distances

Treatment Group	Concentration (ppm)	Kosambi Map Distance (X)											
		Brood											
		1st			2nd			3rd			4th		
		Chromosomal Segment			Chromosomal Segment			Chromosomal Segment			Chromosomal Segment		
		y-cv	cv-v	v-f	y-cv	cv-v	v-f	y-cv	cv-v	v-f	y-cv	cv-v	v-f
Seleno-cystine	0.00	14.13	25.49 26.06	26.06	15.14	27.97 28.47	22.15	19.40	26.43 27.28	27.27	17.26	25.38 25.71	22.04
	0.50	19.67	24.41 24.90	29.32	19.30	22.71 23.55	24.79	21.43	24.91 26.16	21.20	19.51	26.65 27.18	29.87
	1.60	17.29	24.16 24.95	23.56	16.22	29.31 29.62	20.91	20.73	25.53 26.13	27.45	19.67	28.19 28.44	20.96
	5.00	12.97	24.08 24.28	23.10	17.57	25.91 26.35	25.04	24.30	24.28 24.44	18.58	22.96	24.15 24.62	23.38
	25.00	11.95	20.61 20.73	23.08	14.13	24.31 24.87	21.18	12.98	26.52 27.25	21.05	16.09	22.75 23.07	24.27
Seleno-methionine	0.00	14.13	25.49 26.06	26.06	15.14	27.97 28.47	22.16	19.40	26.43 27.28	27.27	17.26	25.38 25.71	22.04
	0.50	18.24	27.60 28.34	25.74	20.28	23.73 24.49	25.73	20.43	26.44 26.85	23.45	28.58	22.23 22.52	29.03
	1.60	18.41	26.67 27.11	23.34	18.18	27.46 28.95	23.70	20.62	22.94 23.34	22.14	20.56	29.07 30.13	24.81
	5.00	15.13	27.95 28.51	27.19	21.86	21.83 22.03	25.70	19.59	24.28 24.36	25.62	18.61	27.05 27.31	23.48
Gluta-thione	0.00	14.13	25.49 26.06	26.06	15.14	27.97 28.47	22.16	19.40	26.43 27.28	27.27	17.26	25.38 25.71	22.04
	-yst (4/5)	14.10	23.05 22.89	24.07	16.20	24.15 24.82	18.81	17.18	21.66 22.20	23.41	20.71	23.06 23.29	25.78
	1000.00	13.98	23.98 24.70	23.46	17.15	22.76 23.38	25.87	16.15	25.30 25.96	25.96	18.39	21.80 22.37	24.85

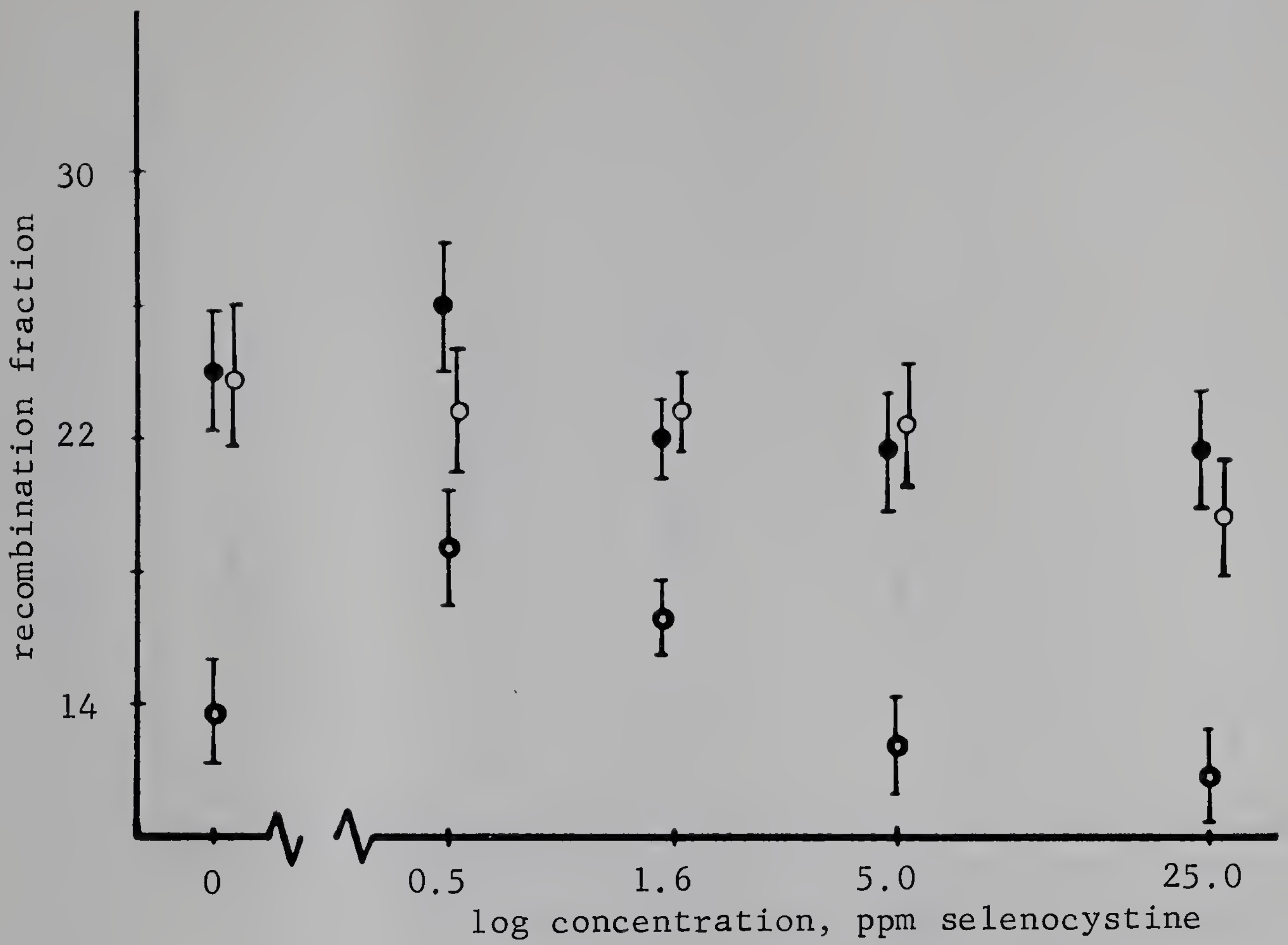


Fig. II-1a. Changes in recombination fractions with varying concentrations of selenocystine, brood 1. (✪ y-cv segment; ○ cv-v segment; ● v-f segment).

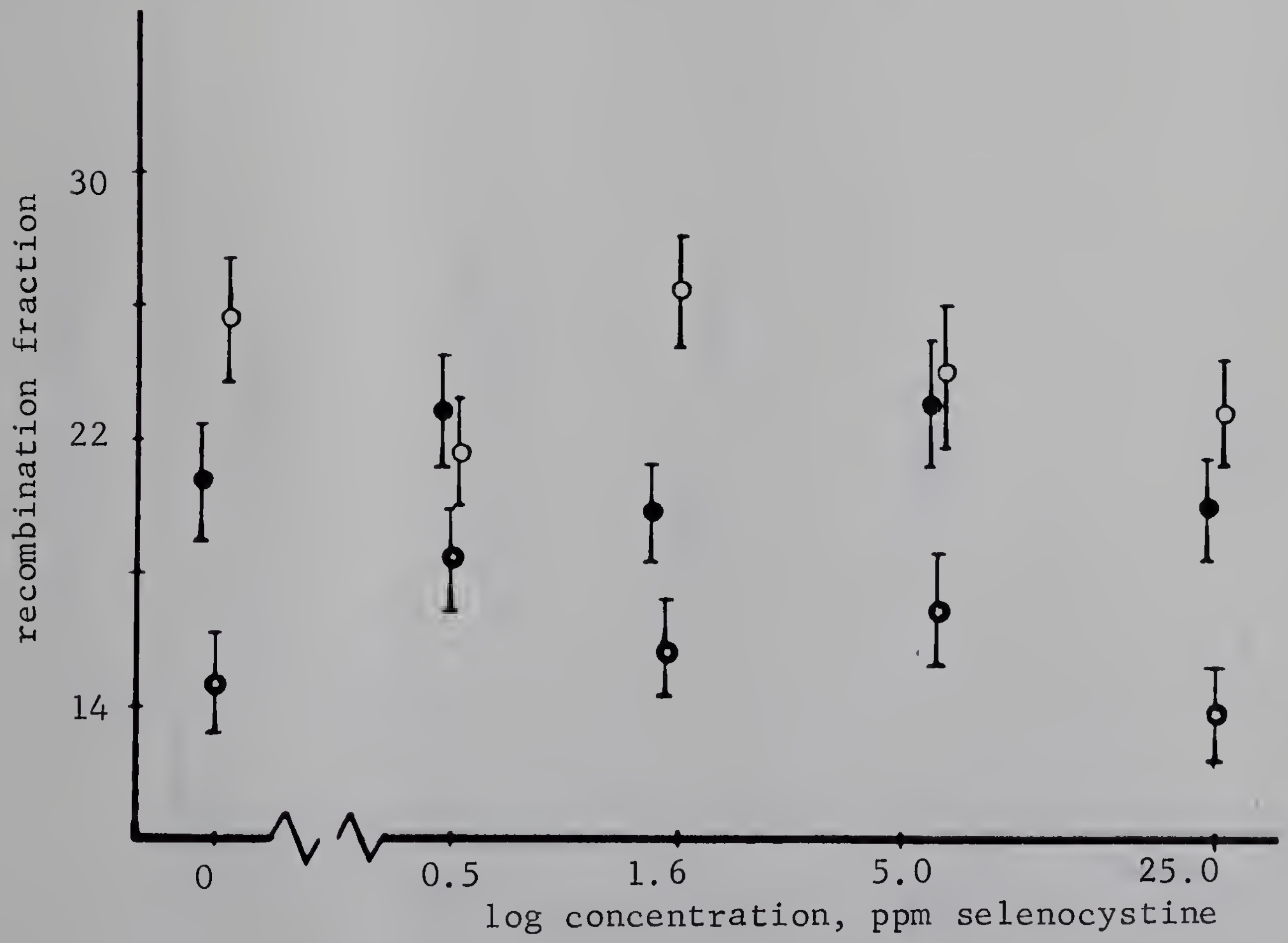


Fig. II-1b. Changes in recombination fractions with varying concentrations of selenocystine, brood 2. (✪ y-cv segment; ○ cv-v segment; ● v-f segment).

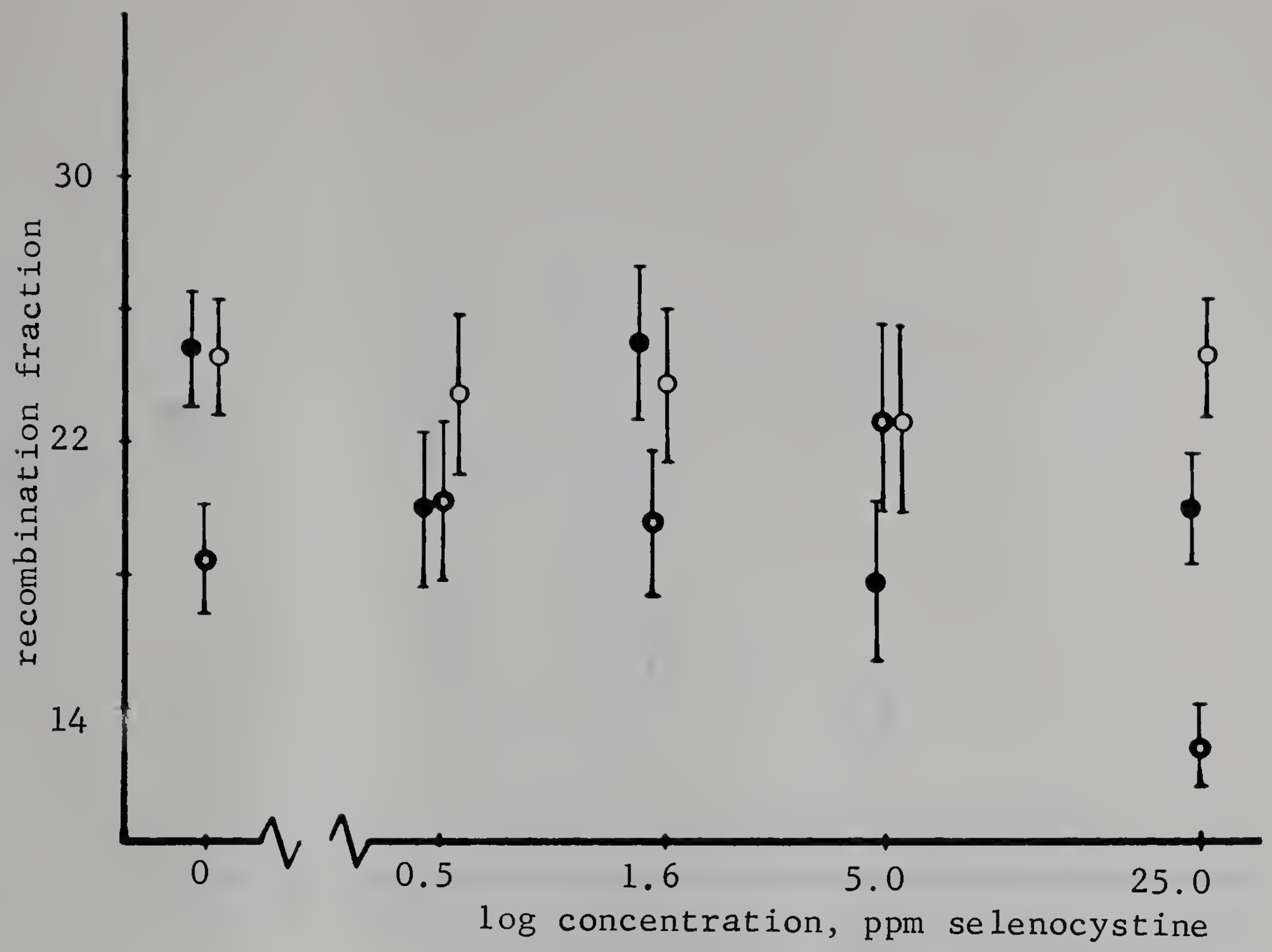


Fig. II-1c. Changes in recombination fractions with varying concentrations of selenocystine, brood 3. (✪ y-cv segment; ○ cv-v segment; ● v-f segment).

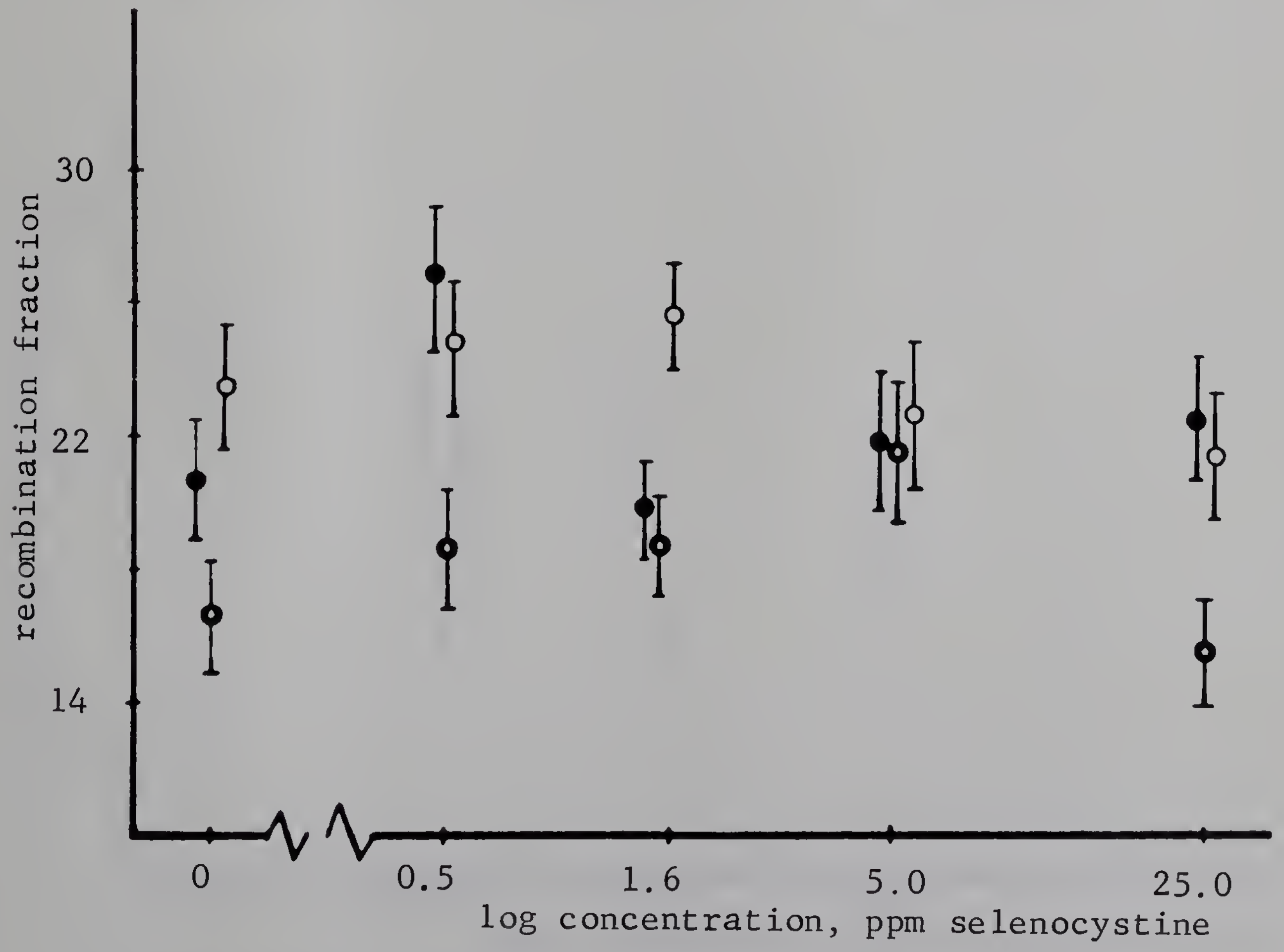


Fig. II-1d. Changes in recombination fractions with varying concentrations of selenocystine, brood 4. (✪ y-cv segment; ○ cv-v segment; ● v-f segment).

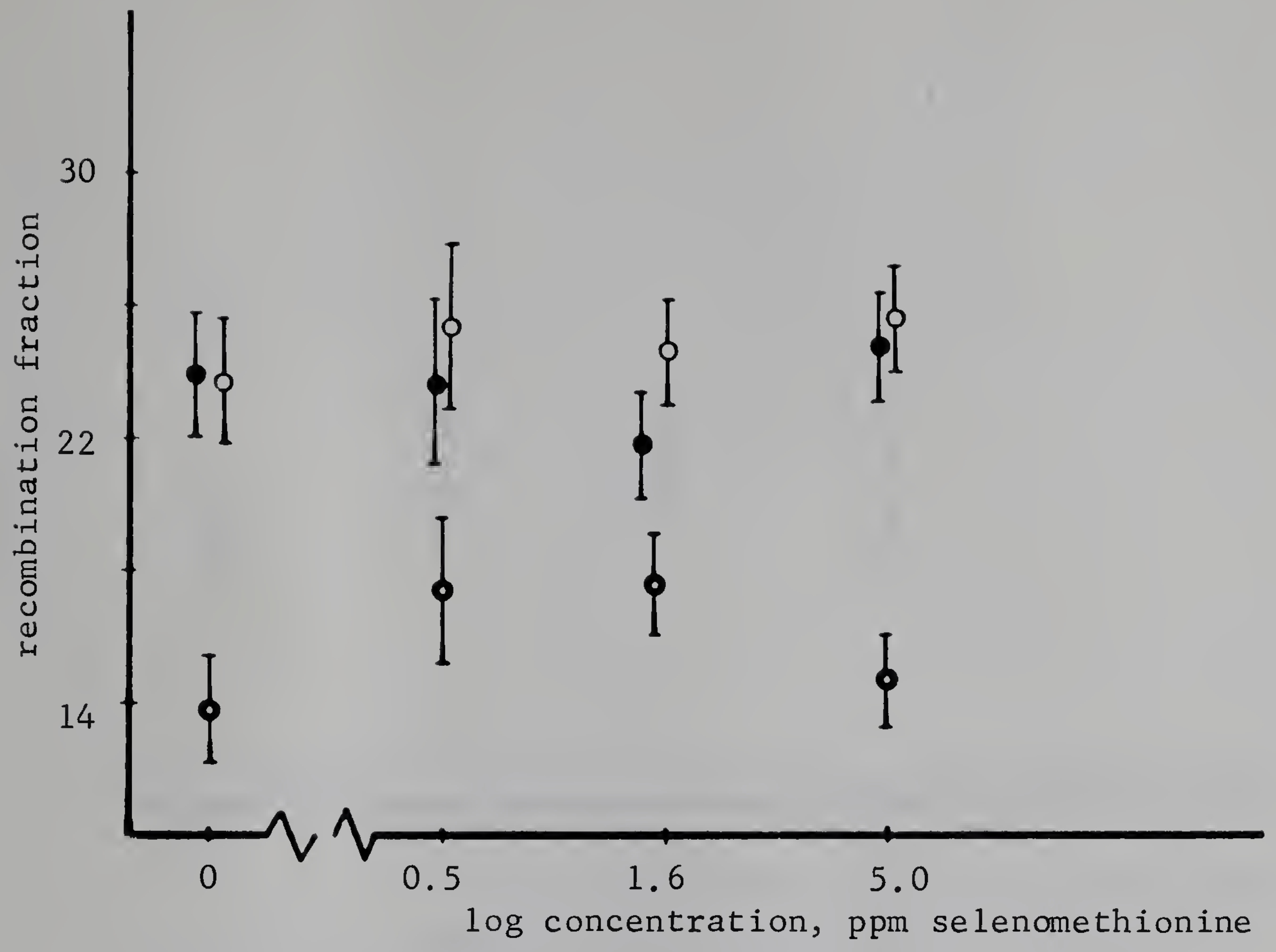


Fig. II-2a. Changes in recombination fractions with varying concentrations of selenomethionine, brood 1. (✪ y-cv segment; ○ cv-v segment; ● v-f segment).

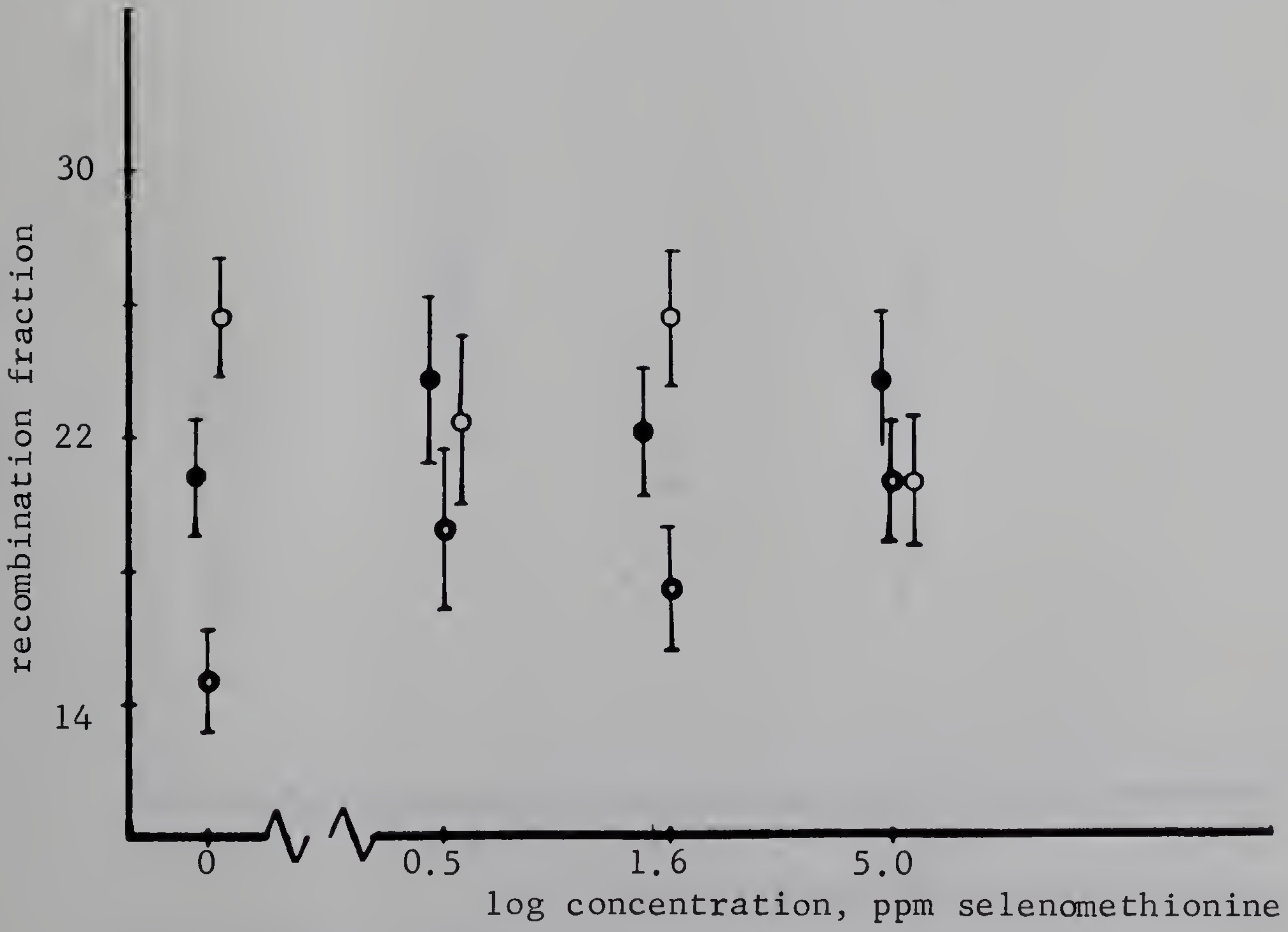


Fig. II-2b. Changes in recombination fractions with varying concentrations of selenomethionine, brood 2. (✪ y-cv segment; ○ cv-v segment; ● v-f segment).

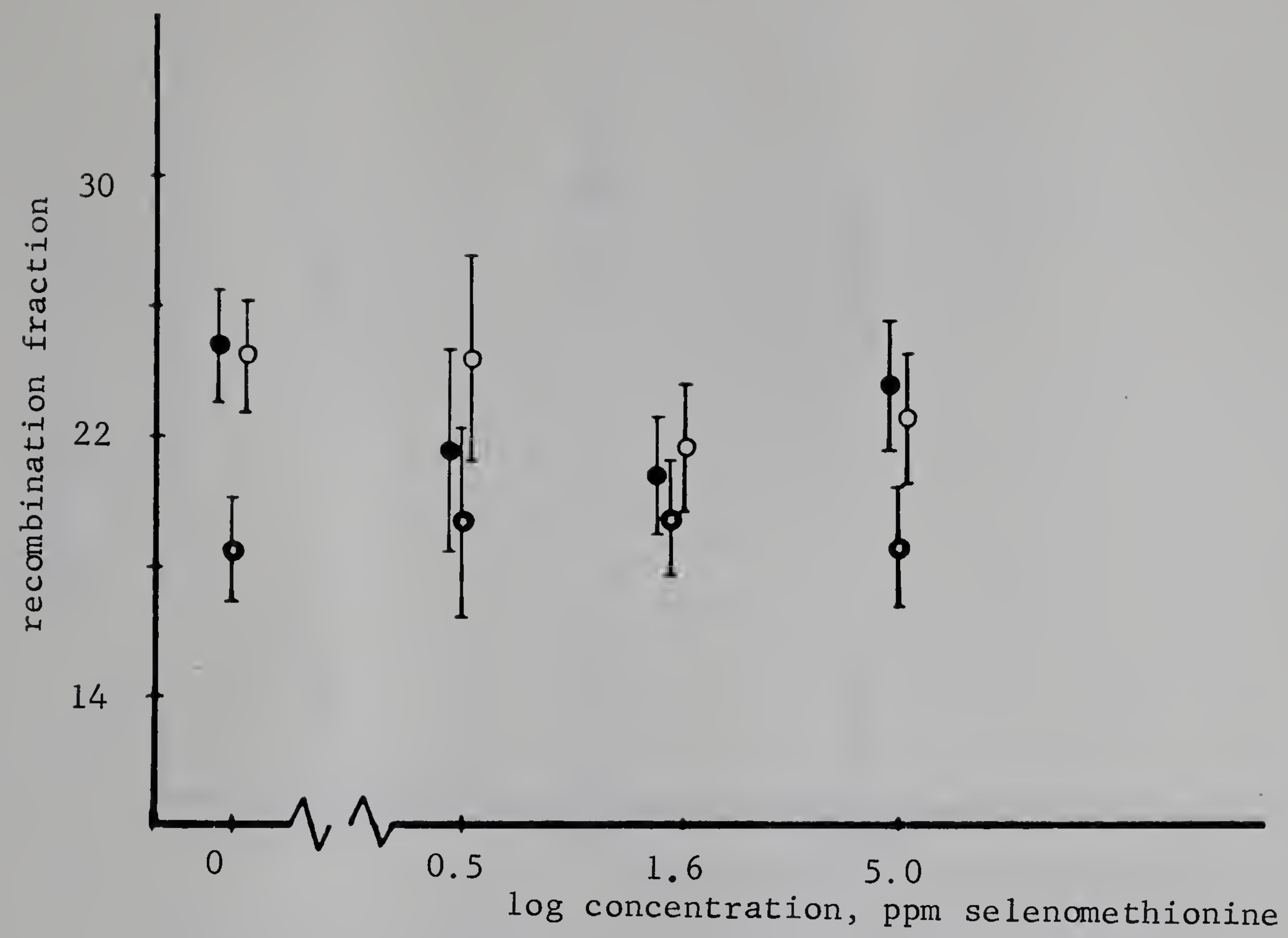


Fig. II-2c. Changes in recombination fractions with varying concentrations of selenomethione, brood 3. (✪ y-cv segment; ○ cv-v segment; ● v-f segment).

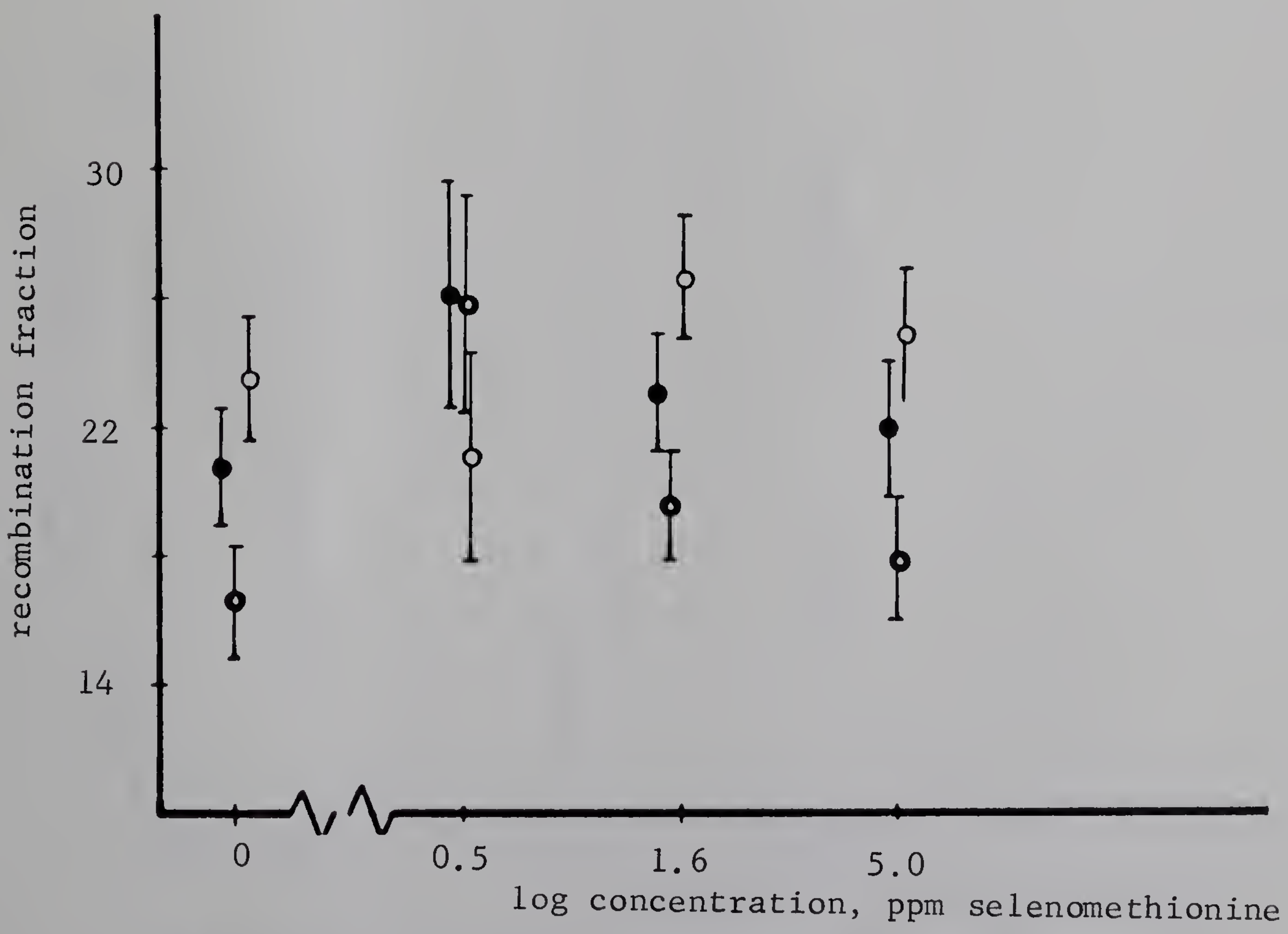


Fig. II-2d. Changes in recombination fractions with varying concentrations of selenomethionine, brood 4. (✪ y-cv segment; ○ cv-v segment; ● v-f segment).

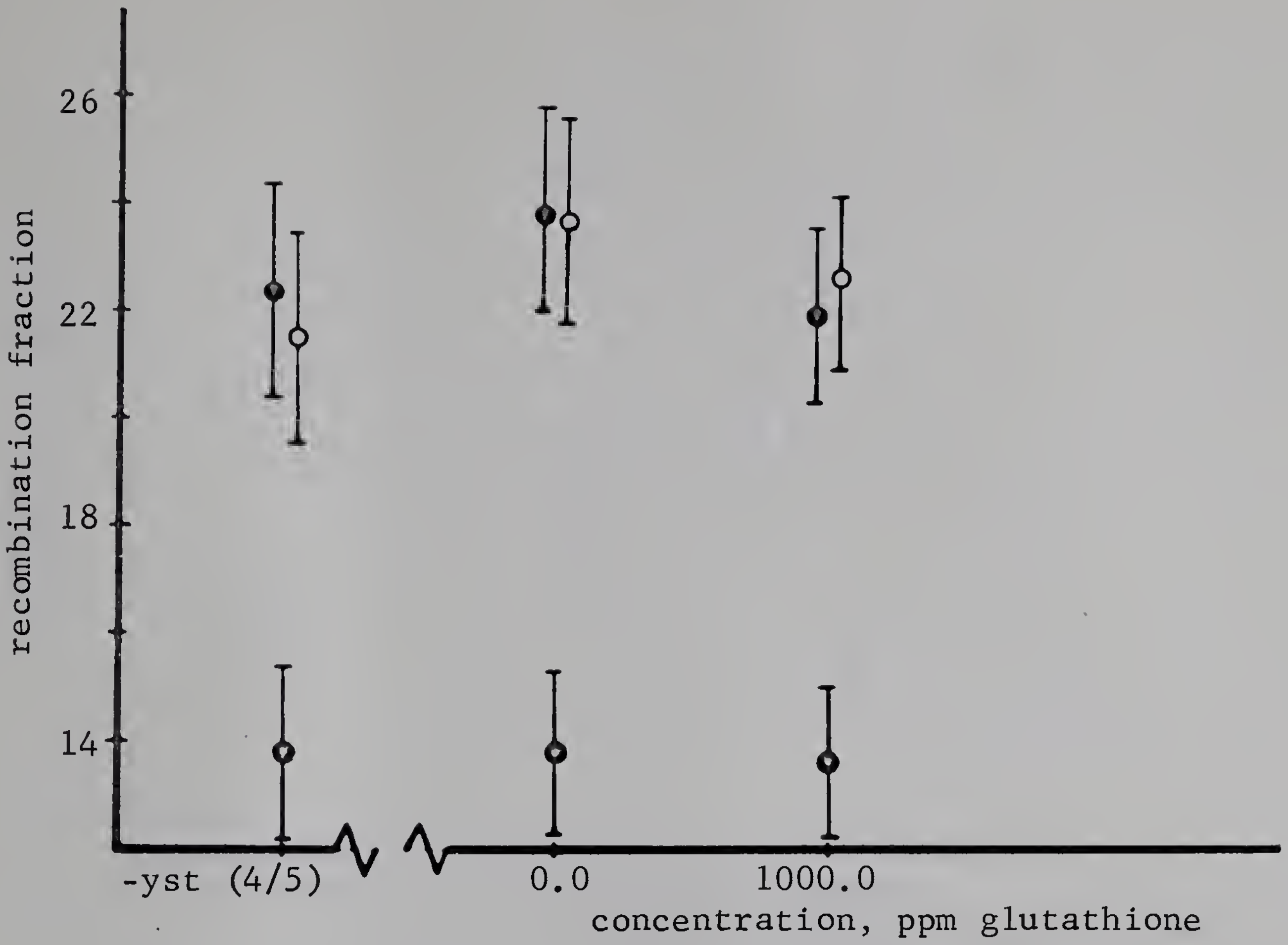


Fig. II-3a. Changes in recombination fractions with varying concentrations of glutathione, brood 1. (✪ y-cv segment; ○ cv-v segment; ● v-f segment).

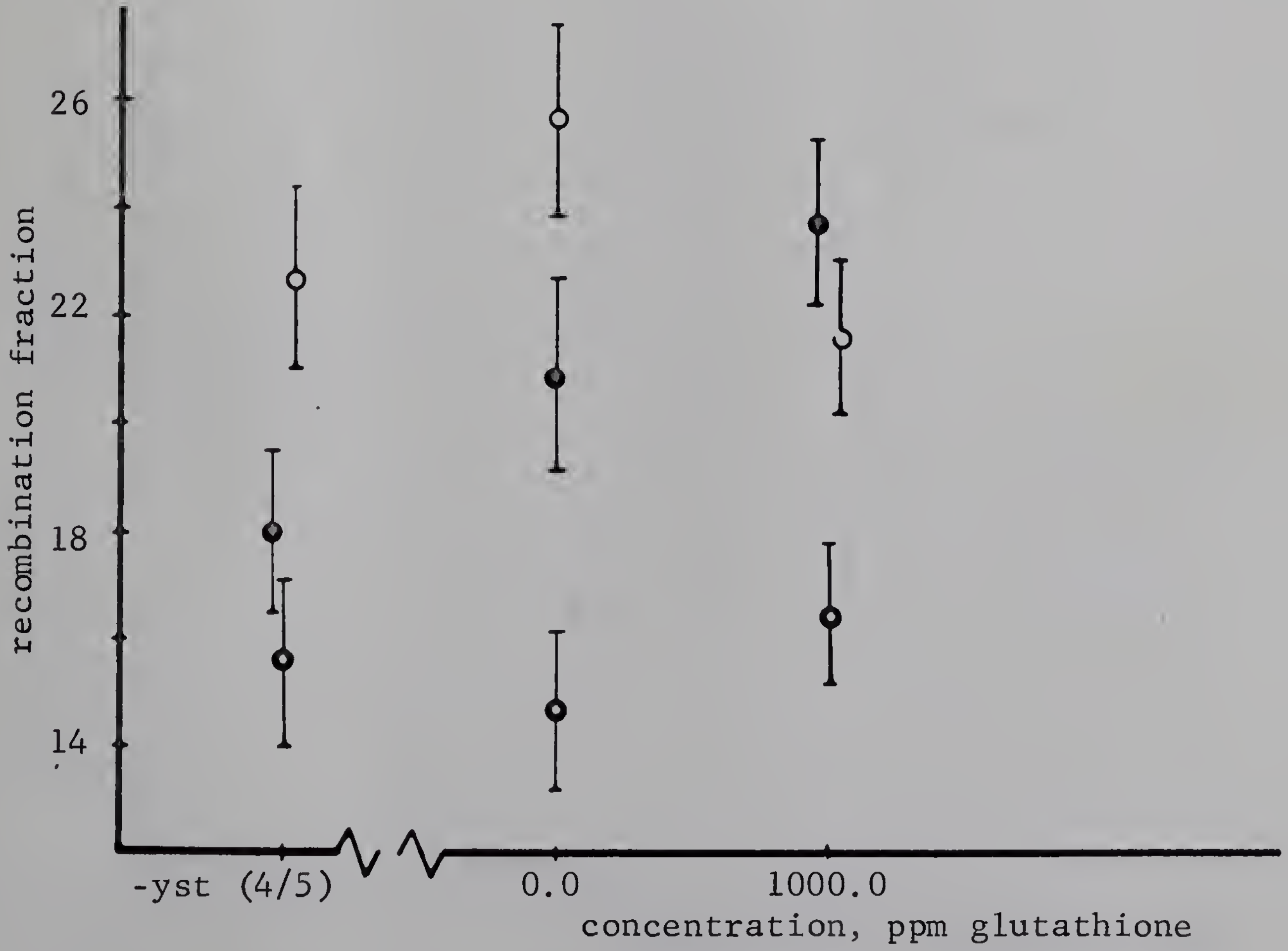


Fig. II-3b. Changes in recombination fractions with varying concentrations of glutathione, brood 2. (✪ y-cv segment; ○ cv-v segment; ● v-f segment).

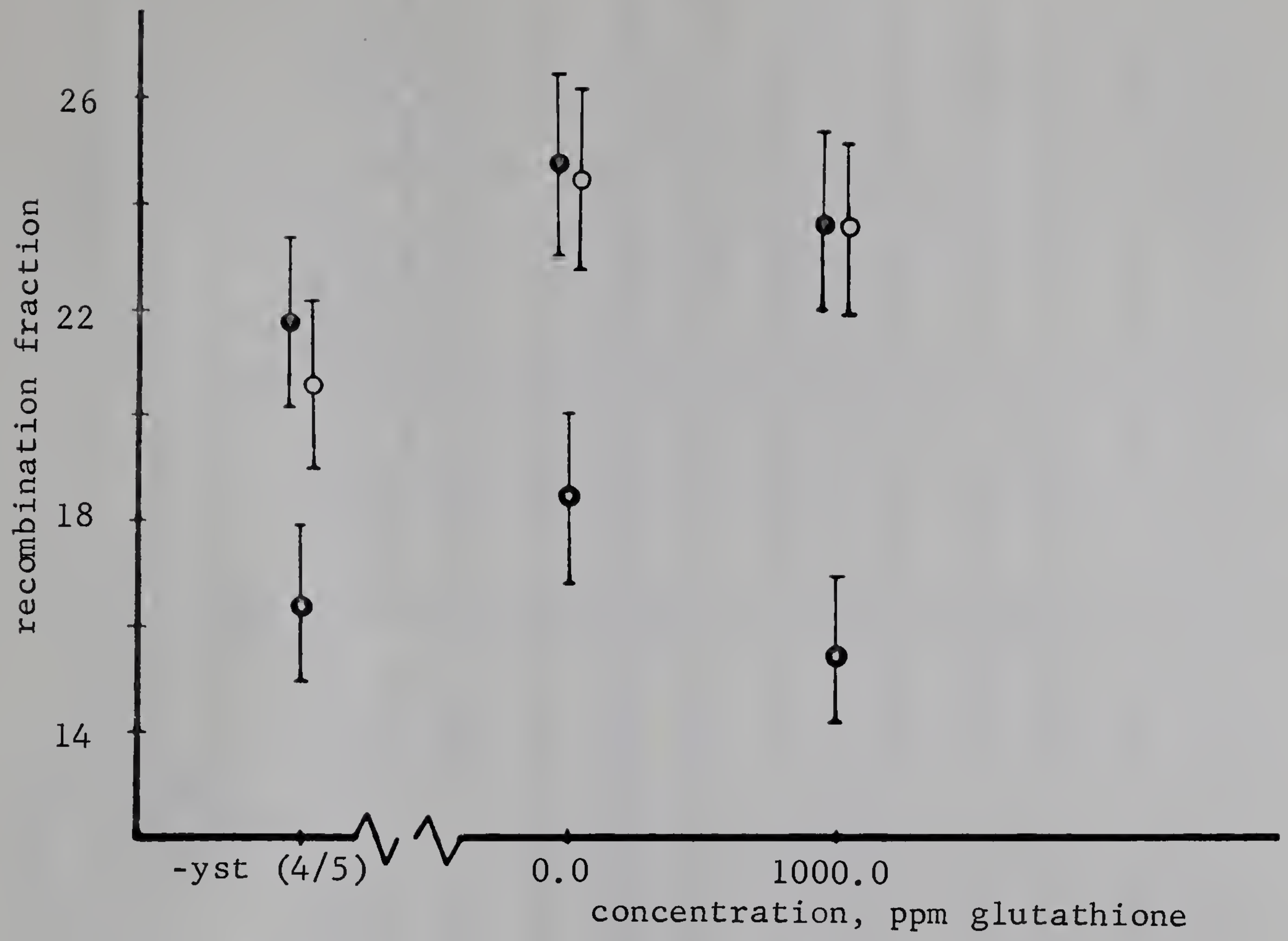


Fig. II-3c. Changes in recombination fractions with varying concentrations of glutathione, brood 3. (✪ y-cv segment; ○ cv-v segment; ● v-f segments).

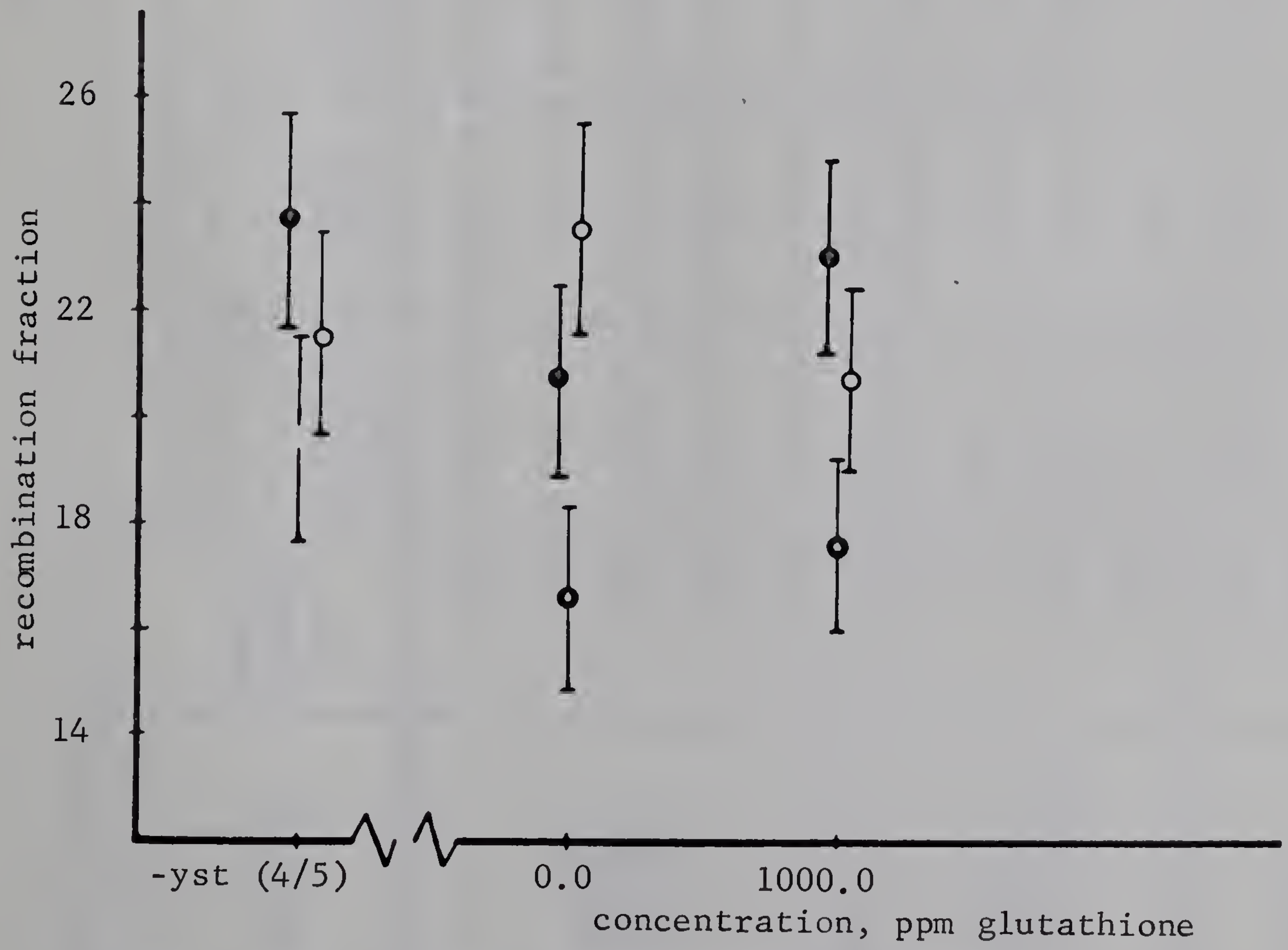


Fig. II-3d. Changes in recombination fractions with varying concentrations of glutathione, brood 4. (✪ y-cv segment; ○ cv-v segment; ● v-f segment).

TABLE II-6

Kosambi coefficients

Treatment	Concentration (ppm)	Kosambi Coefficient (K)							
		Brood							
		1st		2nd		3rd		4th	
		Chromosomal Segment		Chromosomal Segment		Chromosomal Segment		Chromosomal Segment	
		y-cv-v	cv-v-f	y-cv-v	cv-v-f	y-cv-v	cv-v-f	y-cv-v	cv-v-f
Seleno-cystine	0.00	0.42±0.17	0.93±0.19	0.39±0.14	0.77±0.17	0.28±0.11	0.87±0.16	0.37±0.15	0.65±0.18
	0.50	0.59±0.03	1.02±0.20	0.17±0.09	1.06±0.20	0.14±0.10	1.01±0.29	0.43±0.15	0.80±0.17
	1.60	0.37±0.10	0.93±0.15	0.43±0.12	0.64±0.13	0.51±0.19	0.99±0.25	0.59±0.13	0.75±0.14
	5.00	0.24±0.14	0.53±0.15	0.80±0.23	1.16±0.23	0.53±0.22	0.63±0.28	0.42±0.16	0.79±0.22
	25.00	0.20±0.14	0.52±0.15	0.47±0.16	1.00±0.20	0.21±0.12	0.84±0.19	0.15±0.11	0.51±0.15
Seleno-methionine	0.00	0.42±0.17	0.93±0.19	0.39±0.14	0.77±0.17	0.28±0.11	0.87±0.16	0.37±0.15	0.65±0.18
	0.50	0.24±0.14	0.70±0.20	0.09±0.09	0.68±0.23	0.23±0.16	0.58±0.26	0.84±0.34	1.15±0.41
	1.60	0.40±0.13	0.74±0.15	0.15±0.09	1.09±0.22	0.39±0.14	0.75±0.20	0.35±0.12	0.95±0.19
	5.00	0.38±0.13	0.80±0.14	0.46±0.16	0.66±0.17	0.50±0.18	0.60±0.16	0.68±0.21	0.88±0.22
Gluta-thione (s4)	0.00	0.42±0.17	0.93±0.19	0.39±0.14	0.77±0.17	0.28±0.11	0.87±0.16	0.37±0.15	0.65±0.18
	-yst (4/5)	0.39±0.19	0.36±0.12	0.34±0.14	0.95±0.22	0.22±0.11	0.80±0.19	0.48±0.17	0.70±0.19
	1000.00	0.13±0.09	0.85±0.19	0.16±0.08	0.78±0.15	0.29±0.12	0.85±0.16	0.40±0.15	0.99±0.22

TABLE II-7

Coincidence coefficients

Treatment	Concen- tration (ppm	Coincidence Coefficient (C)							
		Brood							
		1st		2nd		3rd		4th	
		Chromosomal Segment		Chromosomal Segment		Chromosomal Segment		Chromosomal Segment	
		y-cv-v	cv-v-f	y-cv-v	cv-v-f	y-cv-v	cv-v-f	y-cv-v	cv-v-f
Seleno- cystine	0.00	0.31±0.12	0.75±0.12	0.31±0.11	0.63±0.12	0.24±0.08	0.72±0.11	0.29±0.11	0.52±0.13
	0.50	0.45±0.12	0.81±0.12	0.14±0.07	0.80±0.12	0.12±0.08	0.76±0.18	0.35±0.11	0.69±0.12
	1.60	0.29±0.07	0.71±0.09	0.35±0.09	0.54±0.10	0.42±0.14	0.79±0.16	0.49±0.10	0.61±0.10
	5.00	0.18±0.11	0.44±0.11	0.59±0.15	0.88±0.14	0.45±0.16	0.48±0.19	0.35±0.12	0.63±0.15
	25.00	0.13±0.09	0.41±0.11	0.34±0.11	0.75±0.12	0.16±0.09	0.66±0.13	0.11±0.08	0.43±0.12
Seleno- methionine	0.00	0.31±0.12	0.75±0.12	0.31±0.11	0.63±0.12	0.24±0.08	0.72±0.11	0.29±0.11	0.52±0.13
	0.50	0.21±0.11	0.61±0.15	0.07±0.07	0.56±0.16	0.21±0.14	0.49±0.19	0.67±0.23	0.88±0.24
	1.60	0.32±0.09	0.60±0.11	0.13±0.07	0.85±0.14	0.31±0.11	0.58±0.13	0.31±0.09	0.78±0.12
	5.00	0.30±0.09	0.68±0.10	0.36±0.12	0.54±0.12	0.39±0.13	0.51±0.12	0.53±0.15	0.70±0.14
Gluta- thione (s4)	0.00	0.31±0.12	0.75±0.12	0.31±0.11	0.63±0.12	0.24±0.08	0.72±0.11	0.29±0.11	0.52±0.13
	-yst (4/5)	0.27±0.13	0.33±0.11	0.26±0.10	0.69±0.13	0.17±0.08	0.61±0.12	0.38±0.12	0.57±0.13
	1000.00	0.10±0.07	0.66±0.12	0.13±0.06	0.62±0.10	0.23±0.09	0.70±0.11	0.31±0.11	0.74±0.13

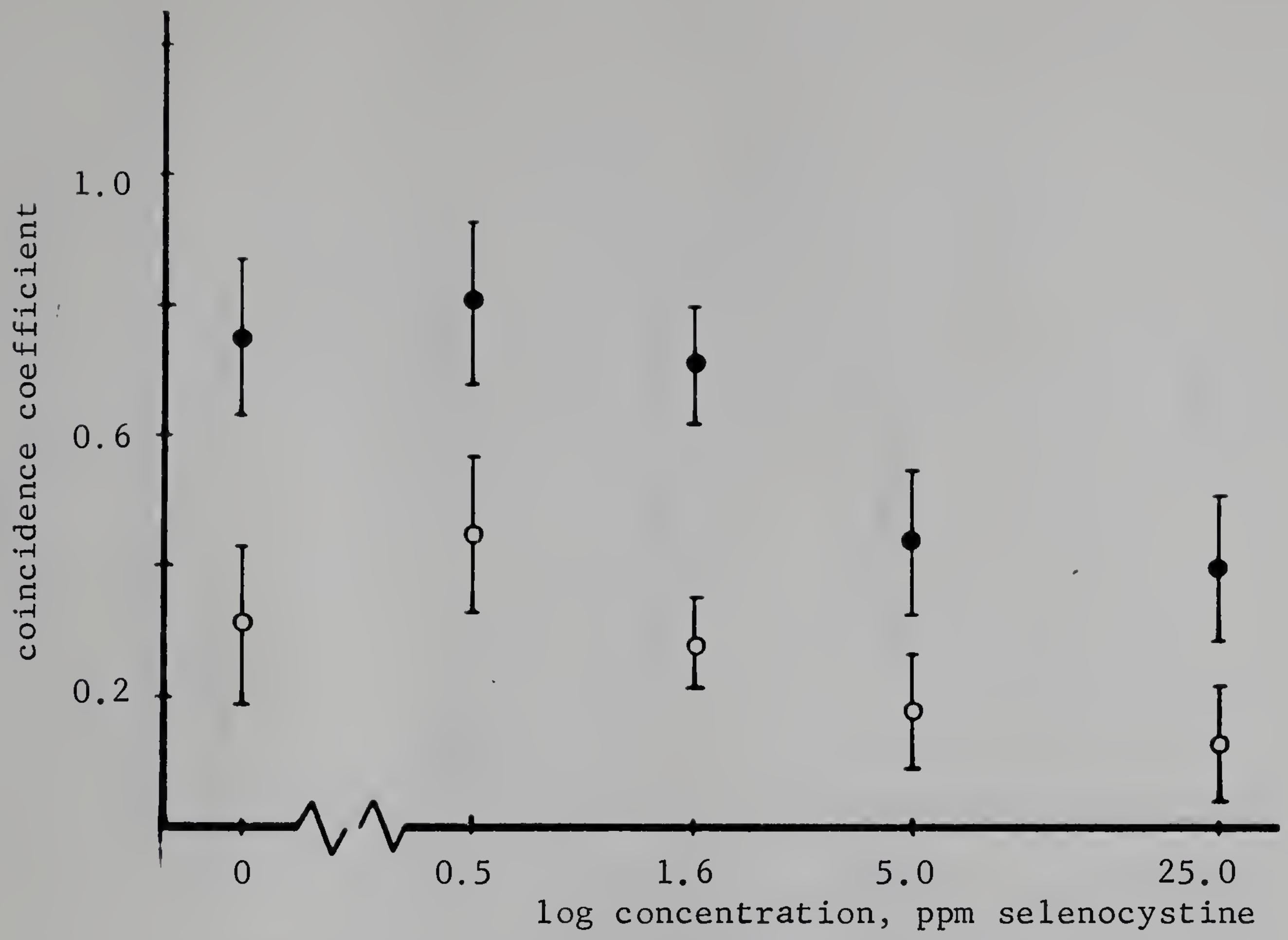


Fig. II-4a. Changes in coincidence coefficients with varying concentrations of selenocystine, brood 1. (○ y-cv-v segment; ● cv-v-f segment).

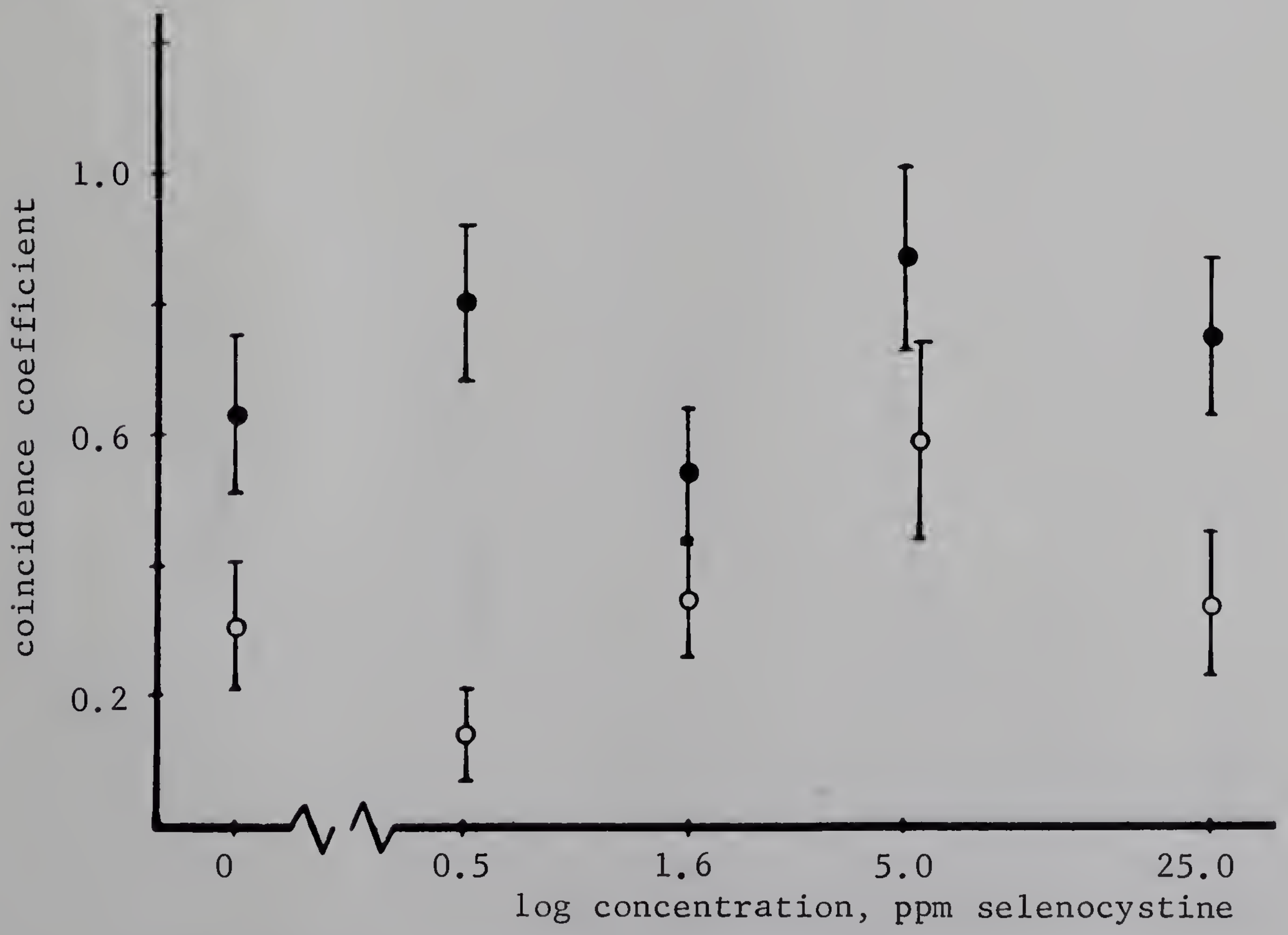


Fig. II-4b. Changes in coincidence coefficients with varying concentrations of selenocystine, brood 2. (○ y-cv-v segment; ● cv-v-f segment).

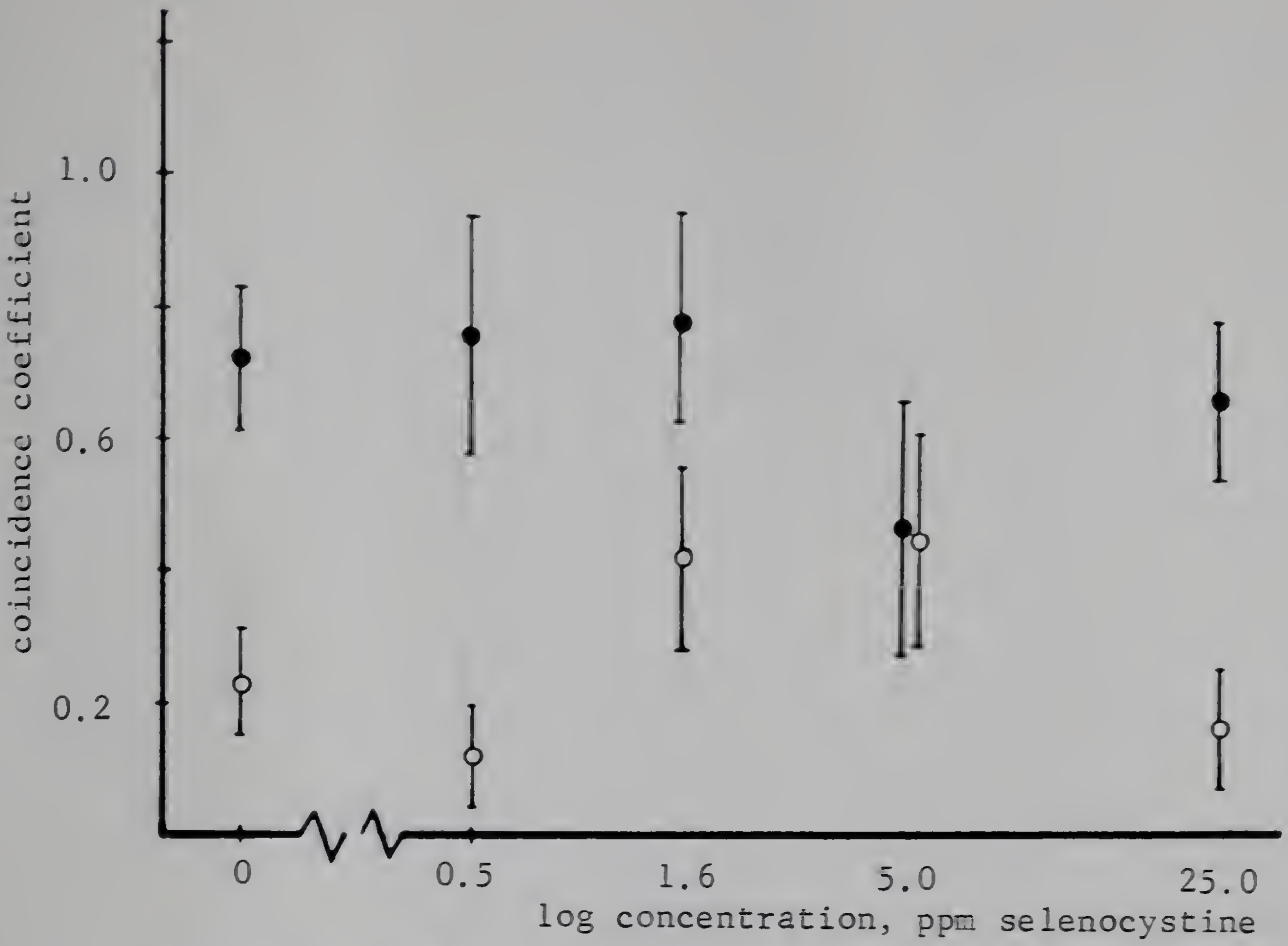


Fig. II-4c. Changes in coincidence coefficients with varying concentrations of selenocystine, brood 3.
(○ y-cv-v segment; ● cv-v-f segment).

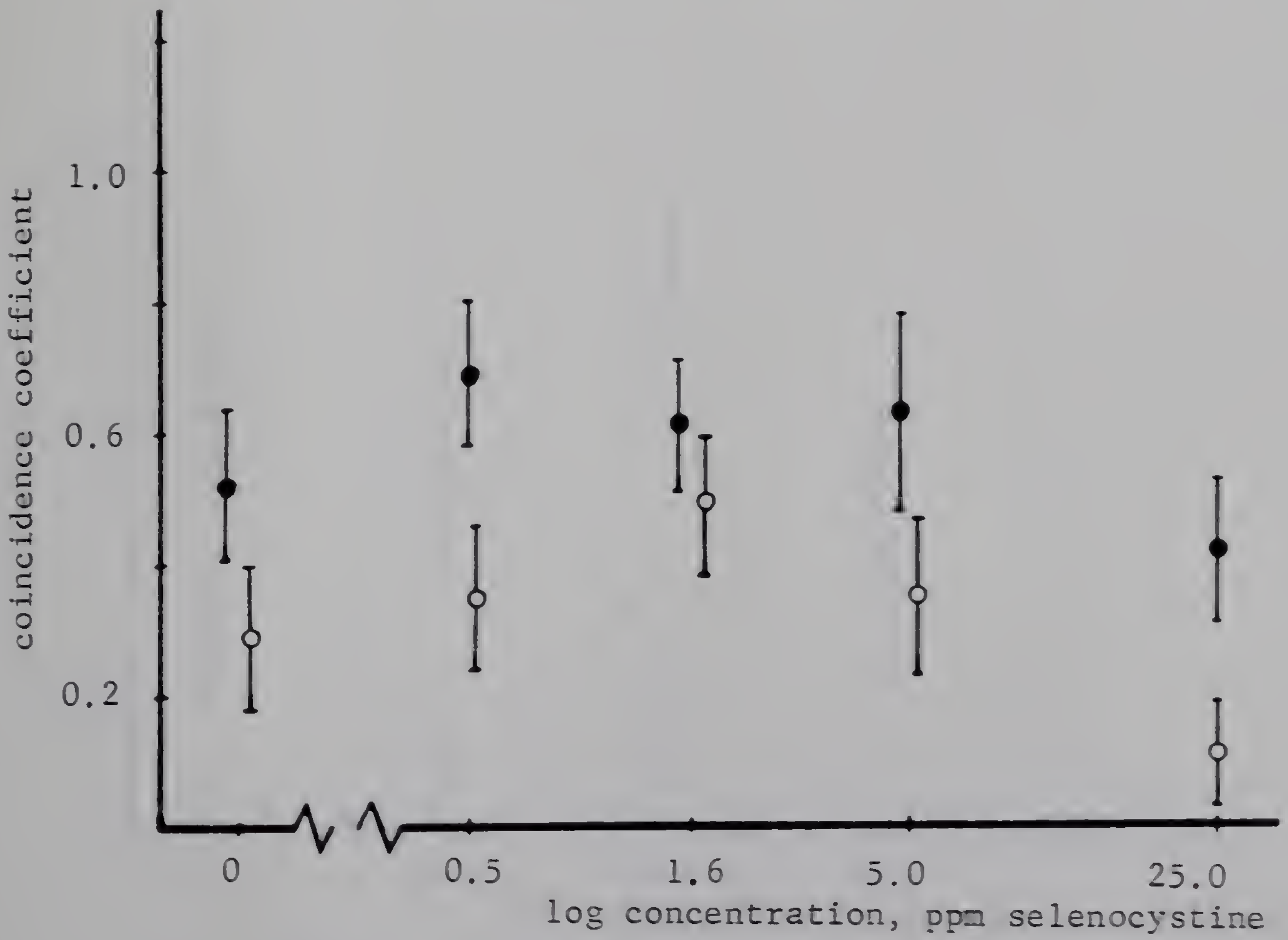


Fig. II-4d. Changes in coincidence coefficients with varying concentrations of selenocystine, brood 4.
(○ y-cv-v segment; ● cv-v-f segment).

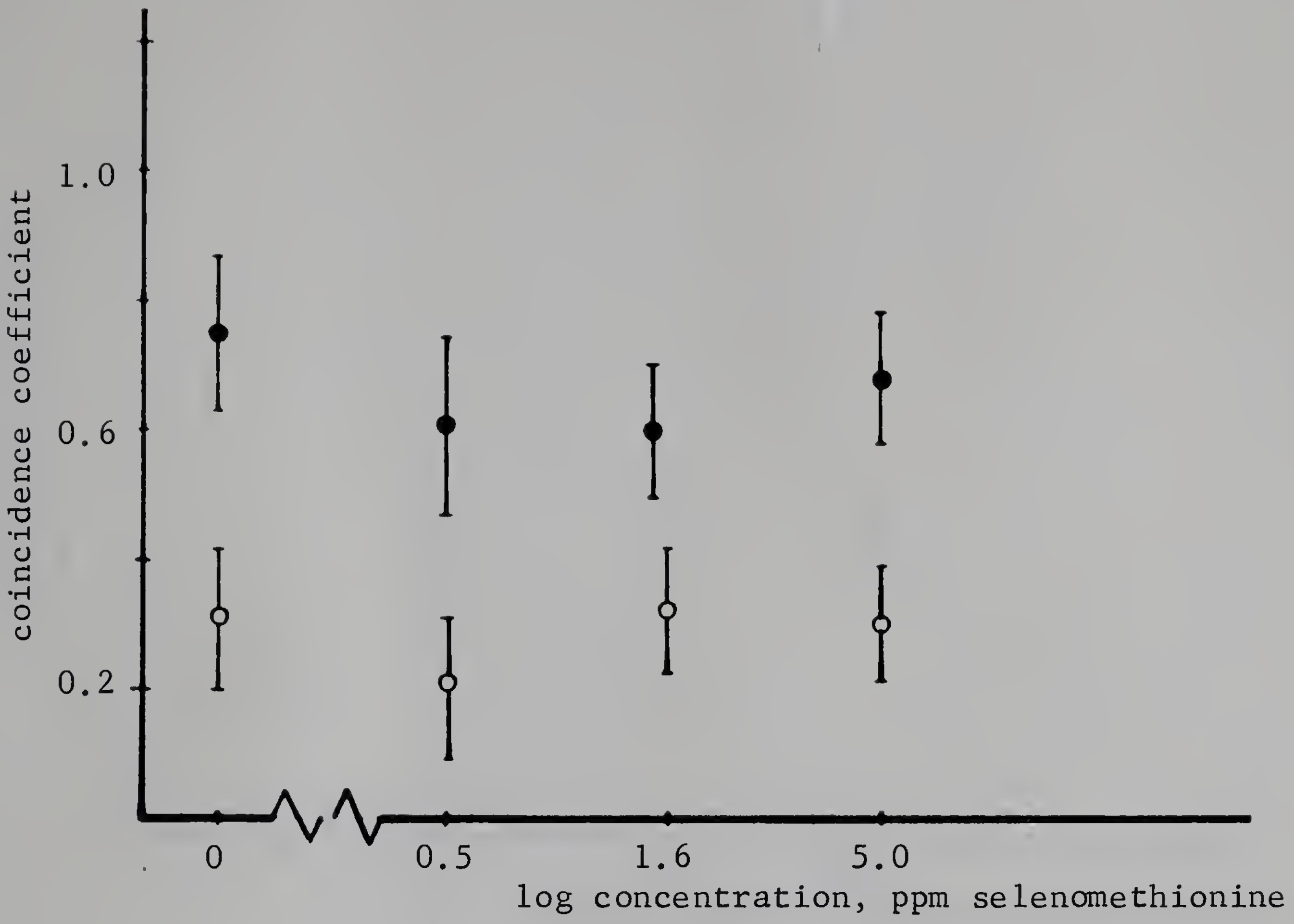


Fig. II-5a. Changes in coincidence coefficients with varying concentrations of selenomethionine, brood 1. (○ y-cv-v segment; ● cv-v-f segment).

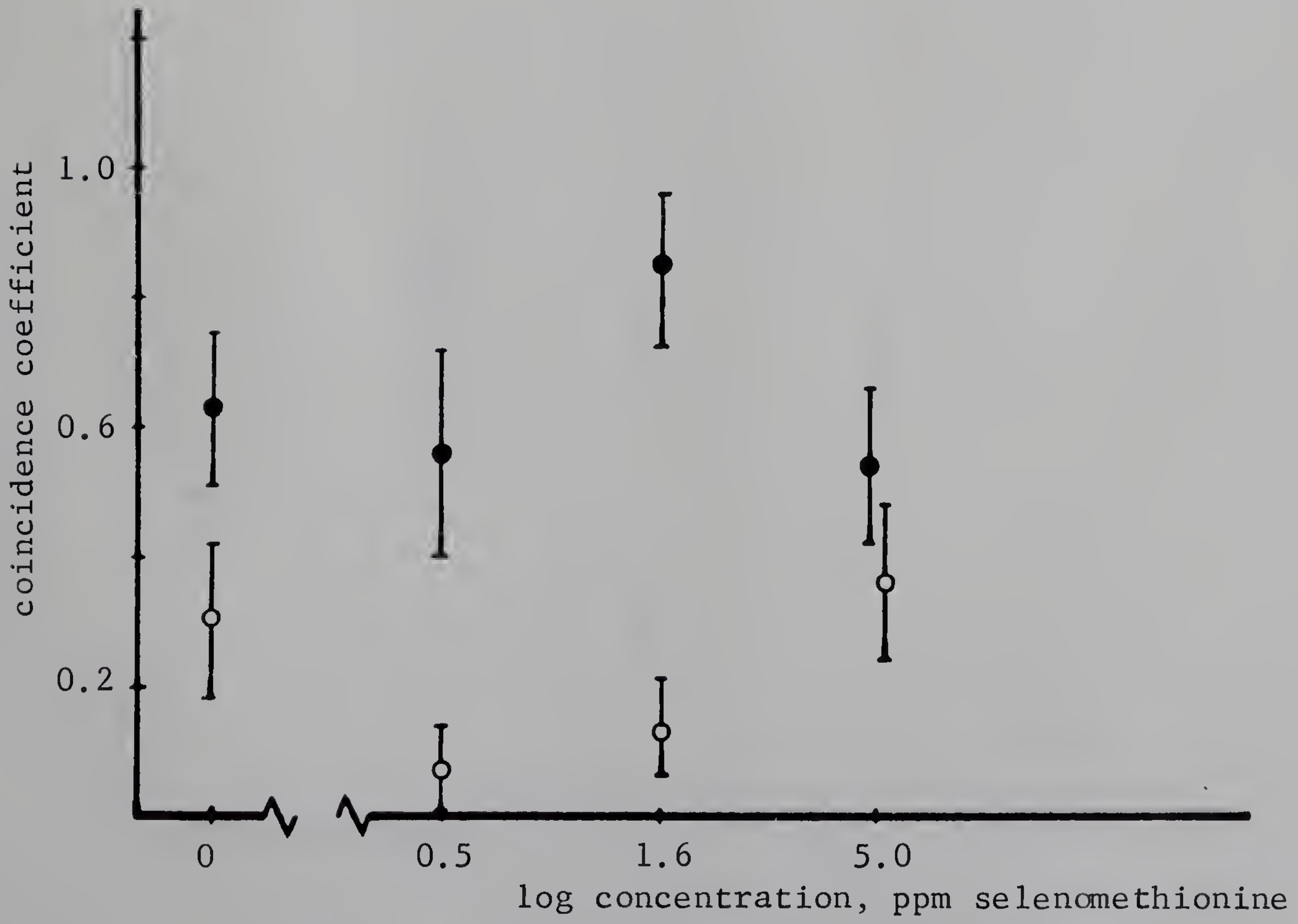


Fig. II-5b. Changes in coincidence coefficients with varying concentrations of selenomethionine, brood 2. (○ y-cv-v segment; ● cv-v-f segment).

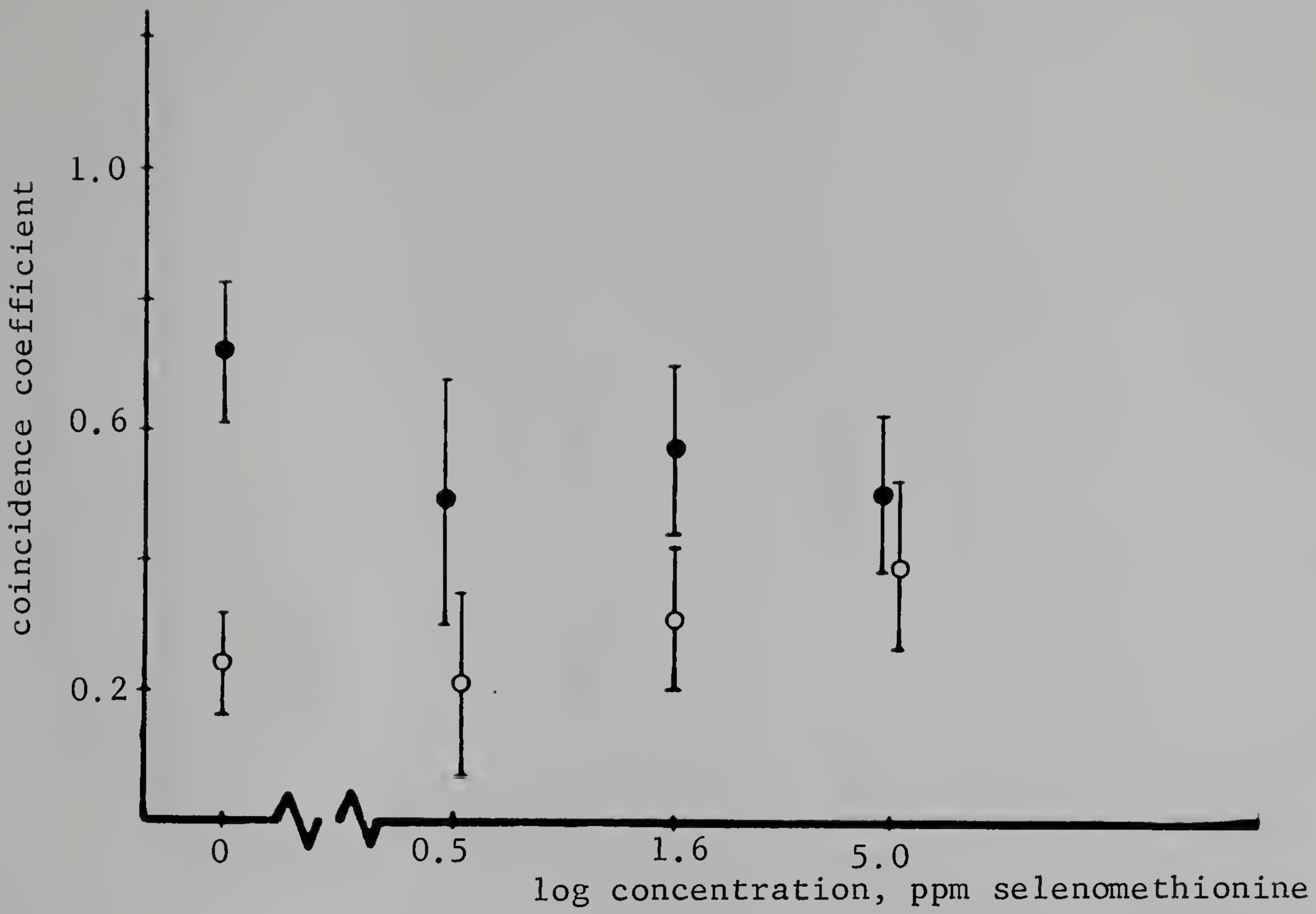


Fig. II-5c. Changes in coincidence coefficients with varying concentrations of selenomethionine, brood 3. (○ *y-cv-v* segment; ● *cv-v-f* segment)

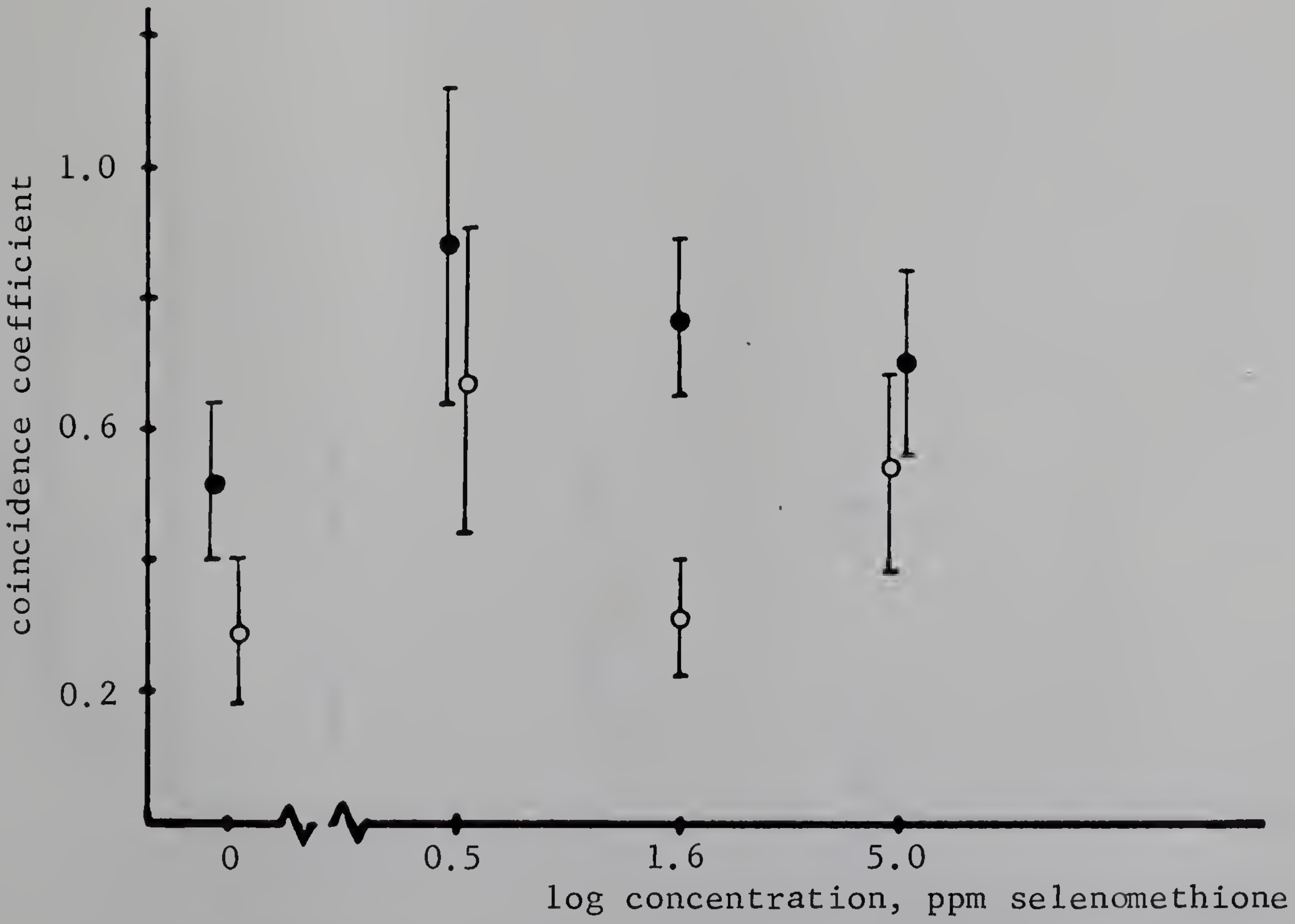


Fig. II-5d. Changes in coincidence coefficients with varying concentrations of selenomethionine, brood 4. (○ *y-cv-v* segment; ● *cv-v-f* segment).

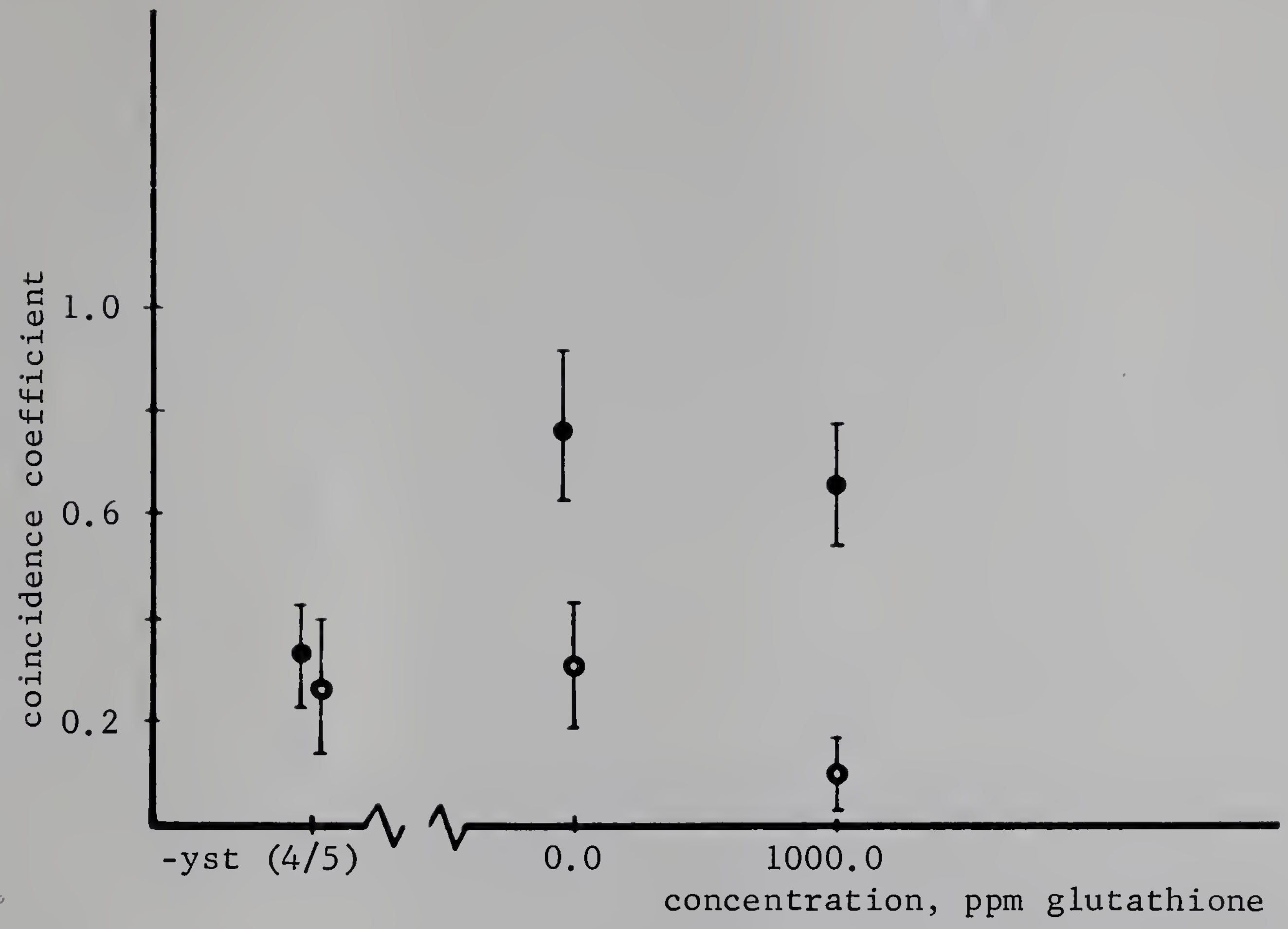


Fig. II-6a. Changes in coincidence coefficients with varying concentrations of glutathione, brood 1. (✪ y-cv-v segment; ● cv-v-f segment).

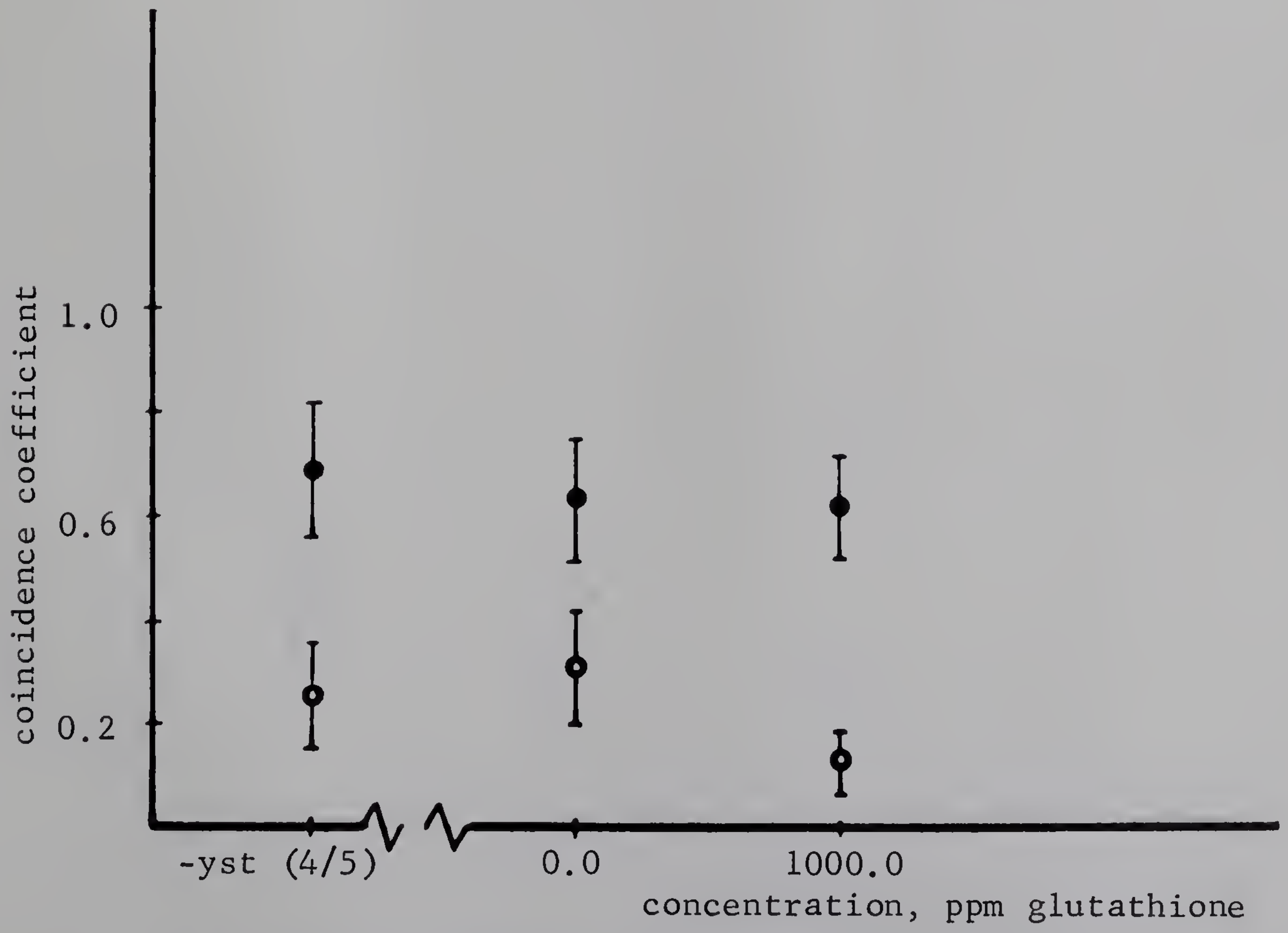


Fig. II-6b. Changes in coincidence coefficients with varying concentrations of glutathione, brood 2. (✪ y-cv-v segment; ● cv-v-f segment).

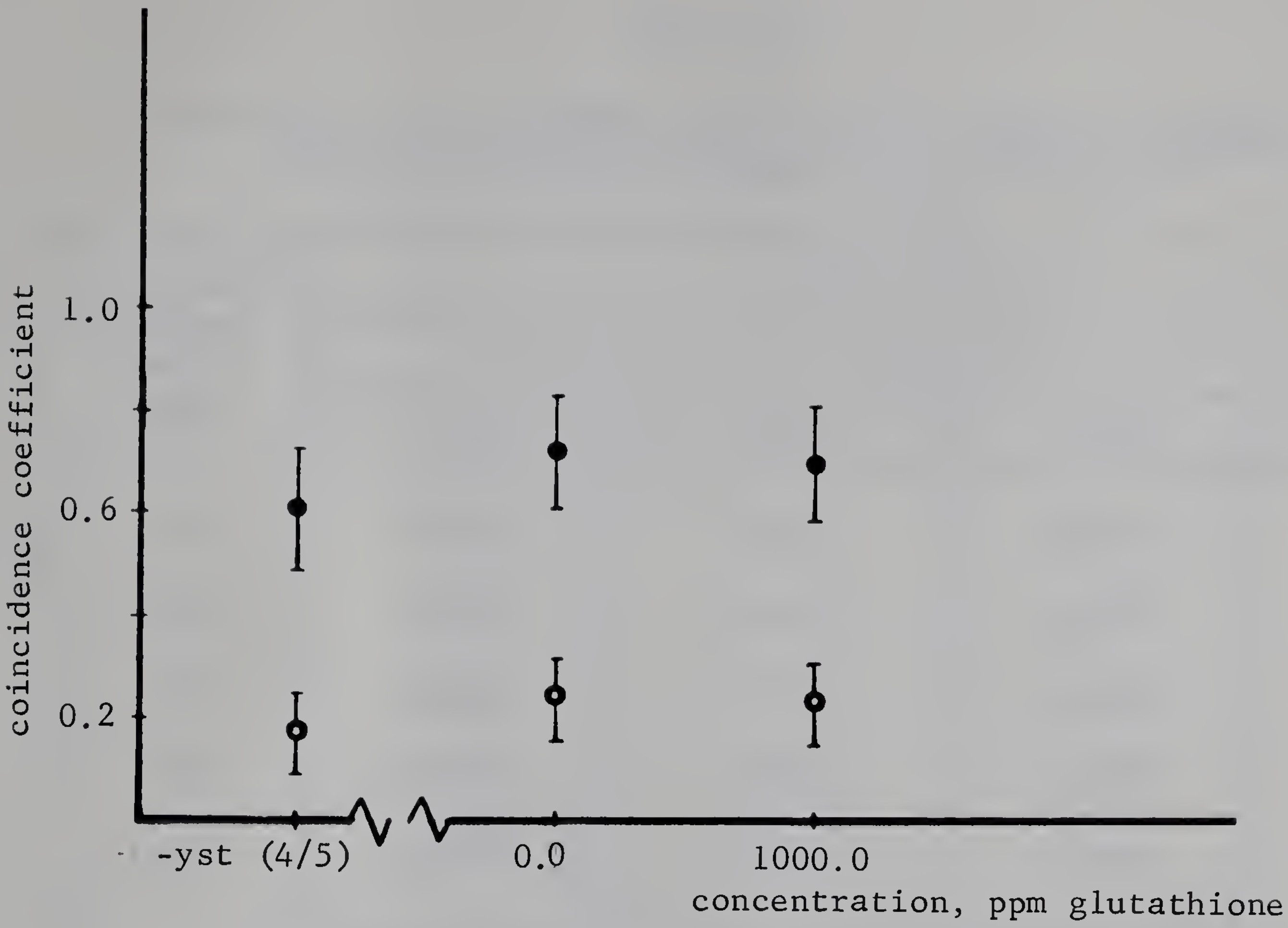


Fig. II-6c. Changes in coincidence coefficients with varying concentrations of glutathione, brood 3. (✪ y-cv-v segment; ● cv-v-f segment).

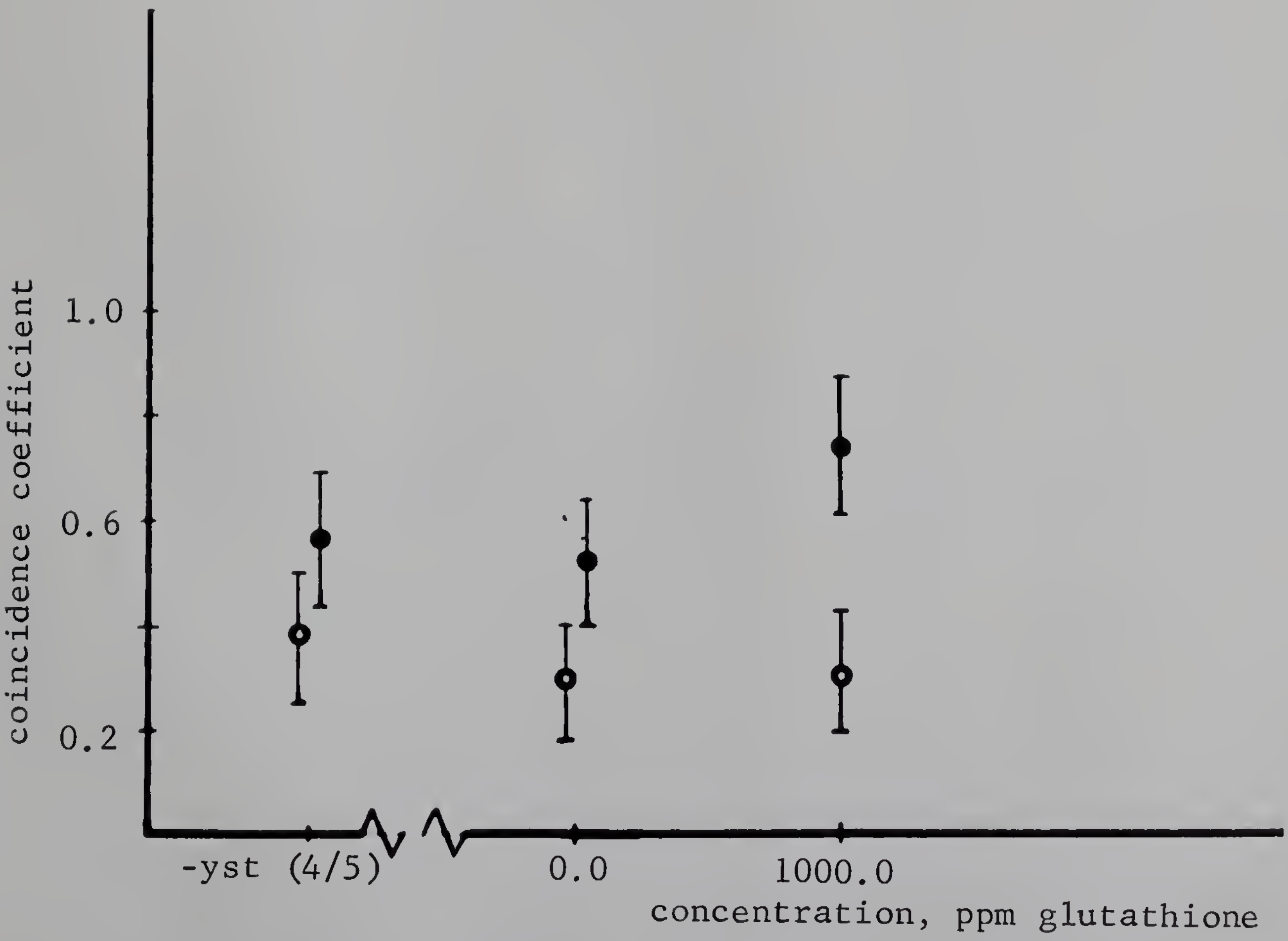


Fig. II-6d. Changes in coincidence coefficients with varying concentrations of glutathione, brood 4. (✪ y-cv-v segment; ● cv-v-f segment).

TABLE II-8

The ratios of the Kosambi coefficients of y-cv-v to cv-v-f for selenocystine treatment ($K_{y\text{-}cv\text{-}v}/K_{cv\text{-}v\text{-}f}$)

Treatment concentration (ppm)	Ky-cv-v/Kcv-v-f			
	Brood			
	1st	2nd	3rd	4th
0.00	0.4452	0.5044	0.3222	0.5698
0.50	0.5756	0.1631	0.1357	0.5324
1.60	0.3984	0.6608	0.5209	0.7863
5.00	0.4489	0.6839	0.8503	0.5263
25.00	0.3866	0.4655	0.2505	0.2917

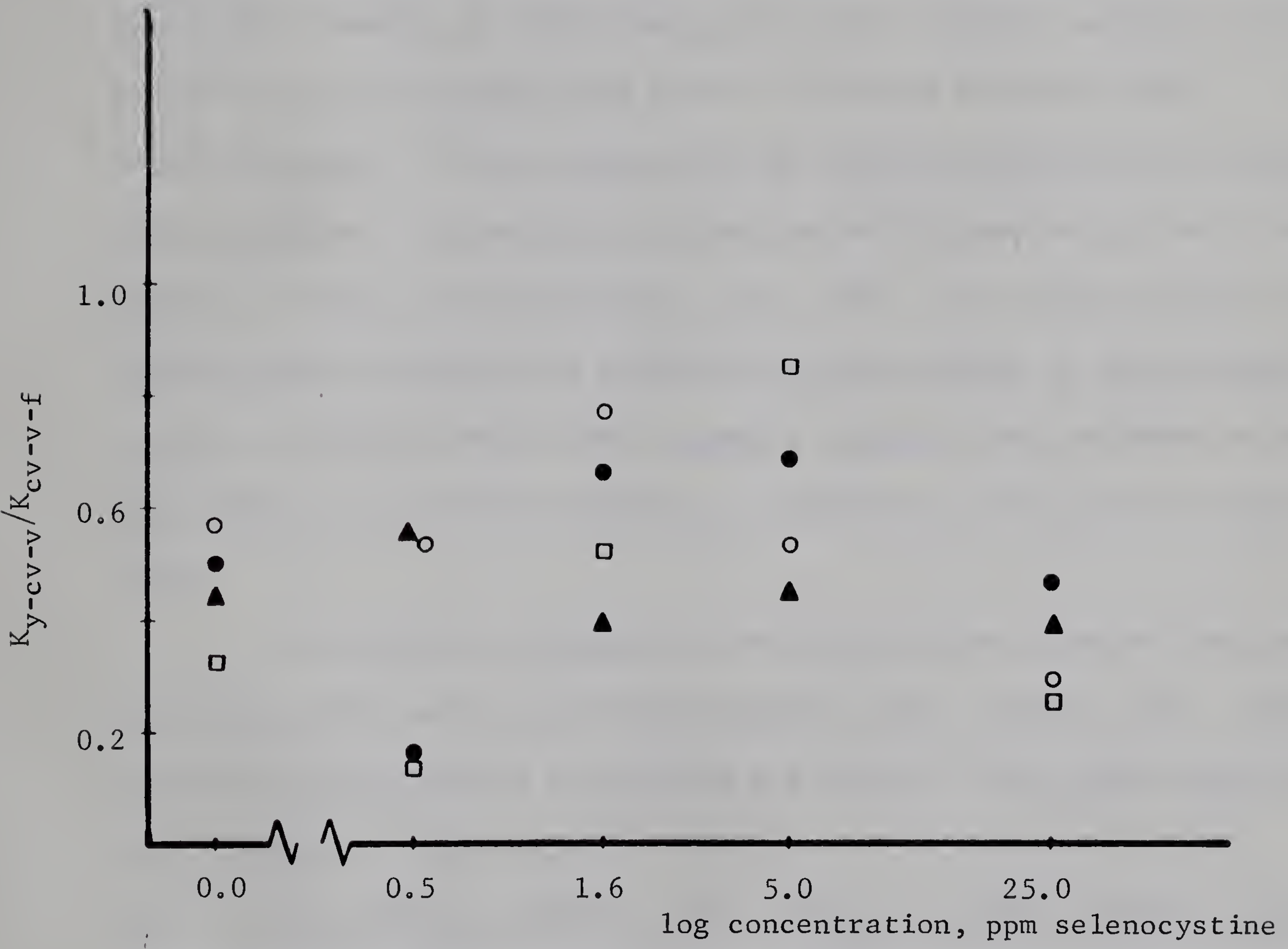


Fig. II-7. Changes in the $K_{y\text{-}cv\text{-}v}/K_{cv\text{-}v\text{-}f}$ ratio with varying concentrations of selenocystine in each brood. (▲ brood 1; ● brood 2; □ brood 3; ○ brood 4).

GENERAL DISCUSSION

Similarities, in the chemical and physical properties, of sulfur and selenium compounds have been recognized for many years (ref. Rosenfeld and Beath 1964). More recently similarities of their physiological properties have been appreciated. Many selenium-fed organisms can incorporate significant amounts of this element into their cellular material, where it is found to be associated with protein (Trelease and Beath 1949). It is significant that, of the products of the enzymatic hydrolysis of seleniferous plant proteins, every amino acid fraction which contains cystine or methionine also contains selenium (Jones et al. 1937). The demonstration that selenocystine is present in tissues of plants growing in the presence of inorganic selenium (Smith 1949) strongly suggests that selenium can substitute for sulfur in the normal processes of formation of the sulfur-bearing amino acids.

In animals, experiments also indicate that selenium- and sulfur-containing amino acids are related metabolically (Painter 1945). Similarities in the behaviour of selenium and sulfur in the animal body have been reported by Moxan et al. (1940).

The genetic analysis of the effects of sodium selenate treatment in barley leads to the conclusion that selenium treatment resulted in a significant reduction in crossing over. Subsequent results of unpublished studies in Ustilago by Dr. C. Person (personal communication) have confirmed this finding.

Cytological examination of the meiotic cells of treated plants, moreover, provides evidence that the reduction of crossing over may be associated with a relaxation of the chromatin content of homologous pairs,

and may not be associated with a failure of homologous pairing.

In the light of this information the experimental findings provide sufficient evidence that protein plays an integral role in the mechanism of crossing over to justify further and more intensive research with regard to this hypothesis. In particular, the question whether the functional protein concerned is structural or enzymatic becomes a valid one. Interference, for which the only explanation given to date is torsion, suggested a test at the molecular level.

Recently it has been suggested that recombination may be mediated by enzymes in phage (Kozinski, Kozinski and Shannon 1963; Mead and Volkin 1964), and in bacteria (Thomasz and Hotchkiss 1964; Howard-Flanders and Boyce 1964). However, Suzuki (1963; 1965) was unable to confirm these suggestions in Drosophila melanogaster using actinomycin D. The question of the existence of such enzymes in higher organisms thus remains open.

Some suggestion that such enzymes might govern a process of DNA repair in nucleoprotein chromosomes as proposed by Whitehouse (1965) has been provided by ultraviolet irradiation of the _ds_ (desynaptic) mutant in barley (Riley, personal communication). If true, this again leads to a need to explain strand breakage, as required by the Whitehouse model. The hypothesis that chromosomal torsion is responsible for this strand breakage is supported by the data obtained by the author for the effects of seleniferous amino acids on crossing over in _Drosophila melanogaster_. It is suggested that the extensive incorporation of selenium into the histone moiety of the nucleoprotein may lessen the degree of torsional strain on the paired homologue, and thus leads to a reduction in crossing over. The following evidence supports the hypothesis:

1. Interference estimates, (coefficients of coincidence) decrease

consistently with the increase of treatment concentration.

2. The intensity of reduction of crossing over differs among segments, being higher in the middle one and lower towards the ends.

However, it would appear probable that, as a result of unexpected increases in both recombination and the coefficient of coincidence at low concentrations of selenocystine treatment, a modification of the interpretation of the above selenium effect is required. Thus, according to the 'local weak site' hypothesis referred to earlier (page 44), the increases mentioned above are the result of localized strand-breakage, which is significant only when the incorporation of selenium is of a low order.

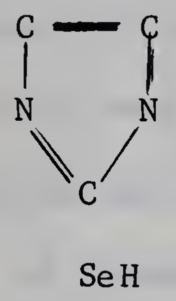
Evidence for a stress imposed on protein molecules by disulfide links between constitutive amino acids, for which diselenides or selenosulfide links could substitute, has accumulated from several studies.

It has been demonstrated that a strain or stress is imposed on the keratin molecule by disulfide bonds, by Rosenthal and Oster (1954). According to these authors, this stress may be relaxed by the occurrence of disulfide-mercaptan conversions in which the mercaptans can be said to act as 'internal plasticizers'. Moreover, evidence has been mounting over the years that disulfide-mercaptan interchange is the process involved in spindle contraction at mitotic anaphase (see Mazia 1958). The liberation of sulfhydryl groups when proteins are denatured may indicate that disulfides hold the protein structure together and that the bonds so formed are ruptured on denaturation (for review see Neurath et al. 1944).

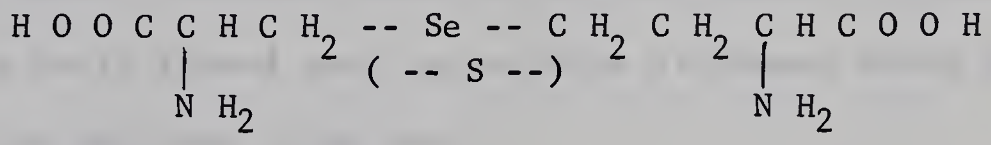
It has been reported further that in contrast to the lability of -SH, protein bound selenium occurs in the reduced state (-SeH), which is oxidized with difficulty (Painter 1940; Nickerson et al. 1956). The effect

of selenium on crossing over found in the present experiments may therefore be explained by a decrease of the stress imposed upon the chromatid strands due to failure of some diselenide-bond formation. This may explain the stretched and dispersed appearance of the selenium-treated meiotic chromosomes shown in Fig. I-1.

In addition to selenocystine and selenomethionine some other kinds of seleno-amino acids have been reported (Painter 1940), e.g.



and



$$HOOC\underset{NH_2}{CH}CH_2 -- \underset{(--S--)}{Se} -- CH_2CH_2\underset{NH_2}{CH}COOH$$

The former is a chemical derivative of imidazole which represents a portion of the histidine molecule that is a major component of histone. Both may have occurred in the chromosomal proteins of sodium selenate treated barley but would unlikely have been present in the selenocystine- and selenomethionine-treated Drosophila.

In view of the many unanswered problems regarding the mechanism of stress-induced breakage, it would be premature to propose a disulfide-mercaptan conversion model to explain it. Whether many or few S S ⟷ S H interconversions would be needed for breakage, whether the stress might be a function of rate or acceleration of such conversions; similarly, whether breakage occurs in the DNA only (because of deformation of the histone) or in the entire nucleoprotein strand, cannot be decided; and again, whether stress might result from coiling or uncoiling, are all matters for speculation. Nevertheless, one suggestion may be made regarding the proposal.

The observation that the distance to which stress-release by a break could extend would depend on the region of the chromosome arm suggests

the corollary that strands should be relatively elastic, for in an inelastic strand relaxation would be distributed over the whole arm.

A possible explanation of the selenium effect on crossing over that has not been discussed is that incorporation of selenium might affect enzyme acceptor sites on the chromosomal protein for a recombinase, and hence affect recombination. This seems an unlikely hypothesis in view of the present data regarding interference. For, although a localized structural alteration might possibly affect neighbouring sites in this manner, it seems unlikely that such effects would extend over appreciable distances along the chromosome as they have been observed to do here.

If Whitehouse is correct, breakage (by either stress or enzyme) would provide a rather more comprehensive hypothesis for both allelic and non-allelic crossing over. Moreover, should further confirmation of the various phenomena observed here show them to be common to meiotic phenomena in organisms with nucleoprotein chromosomes, it might be expected that protein-directed studies may provide some major clues to the understanding of the physiology of meiosis.

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